DOCTOR OF MEDICINE

The effect of spironolactone on exercise capacity in functionally impaired older people without heart failure

Burton, Louise Anne

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THE EFFECT OF SPIRONOLACTONE ON EXERCISE CAPACITY IN FUNCTIONALLY IMPAIRED OLDER PEOPLE WITHOUT HEART FAILURE

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DEGREE OF DOCTOR OF MEDICINE

UNIVERSITY OF DUNDEE

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ABBREVIATIONS

11β-HSD: 11-beta-hydroxysteroid dehydrogenase

6MWT: Six minute walk test

ACE: Angiotensin-converting enzyme

Ach: Acetylcholine

ACT: Anti-chymotrypsin

ACTH: Adrenocorticotrophic hormone

ADL: Activities of daily living

Ang I: Angiotensin I

Ang II: Angiotensin II

ANCOVA: Analysis of covariance

ANP: Atrial natriuretic peptide

ASM: Appendicular skeletal muscle

ATP: Adenosine triphosphate

ATPase: ATP synthase

BDI: Beck depression inventory

BIA: Bioelectrical impedance analysis

BMI: Body mass index

BNP: B-type natriuretic peptide

Ca^{2+}: Calcium

CD: Compact disc

cGMP: Cyclic guanosine monophosphate

CHF: Chronic heart failure

CHQ: Chronic heart failure questionnaire

CI: Confidence interval

COPD: Chronic obstructive pulmonary disease

CRF: Case report form

CRP: C-reactive protein

CSA: Cross-sectional area

CT: Computerised tomography

DHF: Diastolic heart failure

DHPR: Dihydropyridine receptors

DOC: 11-deoxycorticosterone

DXA: Dual energy X-ray absorptiometry

E-C: Excitation contraction coupling

eNOS: Endothelial cell NOS

EQ-5D: EuroQuol questionnaire

EQ-VAS: Visual Analogue Scale questionnaire

ER: Endoplasmic reticulum

ESWT: Endurance shuttle walk test

ETC: Electron transport chain

EWGSOP: European Working Group on Sarcopenia in Older People

FLP: Functional limitation profile

GH: Growth hormone

GHRH: Growth hormone releasing hormone

GM-CSF: Granulocyte-macrophage colony stimulating factor

HADS: Hospital anxiety and depression scale

HCE: Health Care Expenditure

HPA: Hypothalamic-pituitary axis

HRT: Hormone replacement therapy

IADL: Instrumental activities of daily living

ICC: Intra-class correlation

ICIDH: International Classification of Impairments, Disabilities and Handicaps

IGF-1: Insulin-like growth factor

IL-1: Interleukin 1

IL-6: Interleukin 6

iNOS: Inducible NOS

IQR: Inter quartile range

ISWT: Incremental shuttle walk test

JG: Juxtaglomerular

LB: Louise Burton

LV: Left ventricular

LVH: Left ventricular hypertrophy

Mg^{2+}: Magnesium

MHC: Myosin heavy chain

MHRA: Medicines and Healthcare Products Regulatory Authority

MMSE: Mini mental state examination

MR: Mineralocorticoid receptor

MRI: Magnetic resonance imaging

NADPH: Nicotinamide adenine dinucleotide phosphate

NHP: Nottingham health profile

nNOS: Neuronal NOS

NO: Nitric oxide

NOS: Nitric oxide synthase

NYHA: New York Heart Association

OPCS: Office of Population Censuses and Surveys

PIIINP: Procollagen type III amino peptide

PIL: Patient information leaflet

PRT: Progressive resistance training

RAAS: Renin-angiotensin-aldosterone system

RCB: Robertson Centre for Biostatistics

RDA: Recommended dietary allowance

ROC: Reactive oxygen species

RyR1: Ryanodine-sensitive calcium channels

SAE: Serious adverse event

SAR: Serious adverse reaction

SD: Standard deviation

SHBG: Sex hormone binding globulin

SIP: Sickness impact profile

SPCRN: Scottish Primary Care Research Network

SPPB: Short Physical Performance Battery

SPPIR: Scottish Practices and Professionals Involved in Research

SPSS: Statistical package for social sciences

SR: sarcoplasmic reticulum

SSPC: Scottish School of Primary Care

STAI: State trait anxiety score

TGUG: Timed-Get-Up and Go

TLM: Total lean leg mass

TNF-α: Tumour necrosis factor alpha

TSF: Triceps skin fold

U+Es: Urea and electrolytes

UKCRN: United Kingdom Clinical Research Network

VO_2: Maximal oxygen consumption during peak exercise

VDR: Vitamin D receptor

WHO: World Health Organisation

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DECLARATION

I hereby declare that I am the author of this thesis, that all references cited have been consulted by me and that I have carried out the work described in this thesis. The work described in this thesis has not been previously accepted for a higher degree and I have defined the nature of my contribution to the work within the project described in the thesis.

The work contained within this was carried out during my appointment as a Clinical Research Fellow in the Section of Ageing and Health, Department of University of Dundee, between November 2008 and April 2011.

Signed …… Dated……..

SUMMARY

With a growing ageing population decline in physical function has become a major public health issue, as it is associated with disability in later life. Recent evidence suggests that blockade of the renin-angiotension-aldosterone system may have a role in improving physical function in older people.We hypothesised that inhibition of the renin-angiotensin-aldosterone system with spironolactone would improve physical function in older people without heart failure.

In a double-blind, randomised controlled clinical trial 120 participants, aged ≥65 years with functional impairment were randomized to receive 25mg spironolactone or placebo for 20 weeks. The primary outcome was the change in six-minute walking distance over 20 weeks. Secondary outcomes were change in Timed-Get-Up and Go test, Incremental Shuttle Walk Test, measures of health related quality of life (EuroQol health questionnaire and Functional Limitation Profile) and measures of psychological state (Hospital Anxiety and Depression Scale). Outcomes measures were repeated at 10 and 20 weeks.

Participant mean age was 75 years (SD 6), 65/120 (54%) were male. Only 8/120 participants (6.6%) dropped out (5 from the placebo group, 3 from the spironolactone group). Of the 112 participants who completed the study 95% (106/112) remained on medication at 20 weeks. There was no significant change in six minute walking distance at 20 weeks with a -3.2 (95% CI -28.9, 22.5) metres difference between the spironolactone group related to the placebo group (p=0.81). There was however a significant improvement in quality of life at 20 weeks (a secondary outcome) with a rise in EuroQol EQ-5D score of 0.10 (95% CI 0.03, 0.18) in the spironolactone group

relative to the placebo group (p=<0.01). There were no significant changes between groups in the other secondary outcomes. This trial found that spironolactone was safe and well tolerated, but did not improve physical function in older people who did not have heart failure. Quality of life improved, but the biological plausibility and possible mechanisms for this require further study.

1. LITERATURE REVIEW

1.1 Ageing population

We live in an ageing society. The proportion of older people alive today is greater than any other time in history. The number of people aged over 65 years old is predicted to rise from 16% to 23% of the UK population over the next 30 years[1].

Reductions in fertility rates, accompanied by improvements in health care and living conditions, have contributed to this demographic redistribution and to a growing ageing population. Increases in the proportions of older people are being accompanied by a decline in the proportion of younger people under the age of 15 years old. By 2050, the number of people over 60 years old is predicted to exceed the number of young people, worldwide. Data from the Department of Economic and Social Affairs for the United Nations shows that in 1950 the proportion of older people in the world was around 8%. This then rose to 10% in 2000. Estimations for 2050 are projected to reach 21 percent of the world's population[2]. In Scotland, the number of people aged 65 years or over is projected to increase from three quarters of a million in 2000 to 1.2 million in 2031[3].

Age Structure

The age structure of Scotland's population has changed dramatically over the decades, as shown by the change in the population pyramids (Figure 1.1). Scotland appears to be in the advanced stages of demographic transition towards an ageing population. In addition to the natural phases of demographic transition, age structure has also been affected by unexpected events. National statistics produced by the General Register Office for Scotland in 2008 showed a trend in older women (over 75 years) outnumbering men of the same age[4]. This may correlate with the fact that women tend to live longer than men but it may also demonstrate the effects of higher male mortality rates during the Second World War.

The changes in the population pyramids from 1951 to 2001 may be explained by a combination of a reduction in birth rates, increased life expectancy, overall improved health and living improvements along with the added effects of the 'baby boomer' period in the 1960s which has led to Scotland's increasingly ageing population and current change in age structure. The latest population projections for Scotland have suggested that the total population of Scotland will reach a peak by 2031[5]. Although it is speculated that the total population of Scotland will decline slightly in the future, we expect to see a reduction in the numbers of people in the younger age groups and a consequent rise in people in the older age groups. The ageing population changes within Scotland will broadly be in keeping with the rest of Europe, however the proportion of people age >65 years will rise faster in Scotland than the rest of the UK (Figure 1.2)

Figure 1.1 – Population pyramids 1951-2031 (Scottish Executive 2007)[5]

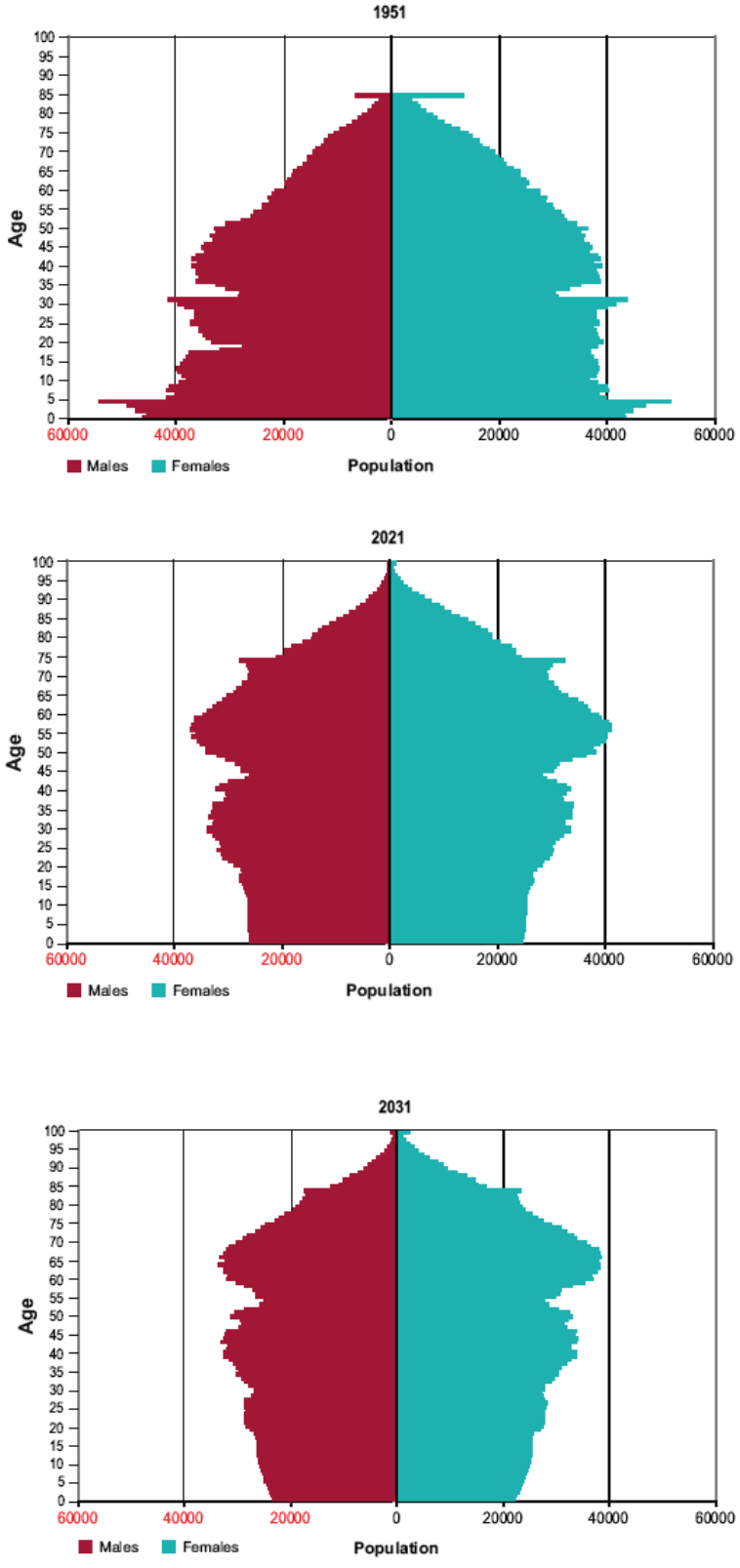
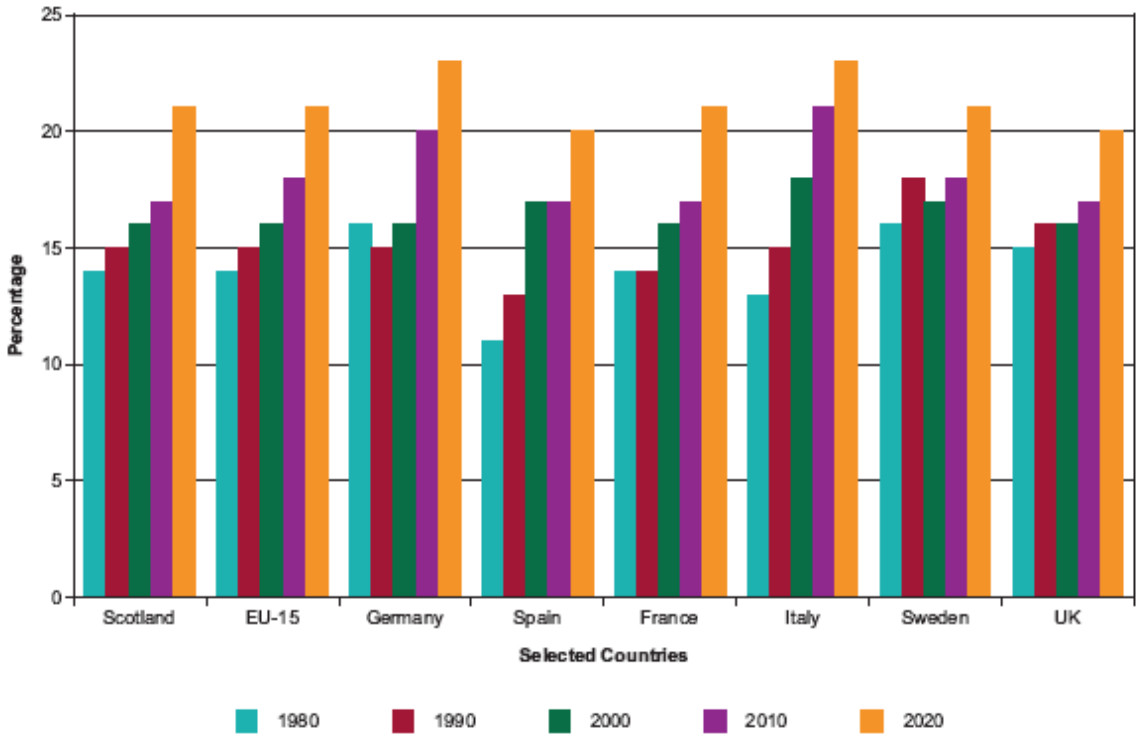


Figure 1.2 – The actual and projected changes in the proportion of the total older population, in Scotland other selected European countries. (Scottish Executive) [5]



EU-15 = 15 countries of the European Union established in January 1995.

(Countries include: Belgium, Greece, Luxembourg, Denmark, Spain, Netherlands, Germany, France, Portugal, Ireland, Italy, United Kingdom, Austria, Finland and Sweden).

Changes in life expectancy

Life expectancy of females in Scotland has risen from 78 years during 1997-1999 to 80 years in 2006 with the gap between males and females narrowing[3]. Despite this improvement in life expectancy it was reported that Scotland's overall life expectancy is poorer than the rest of Western Europe.

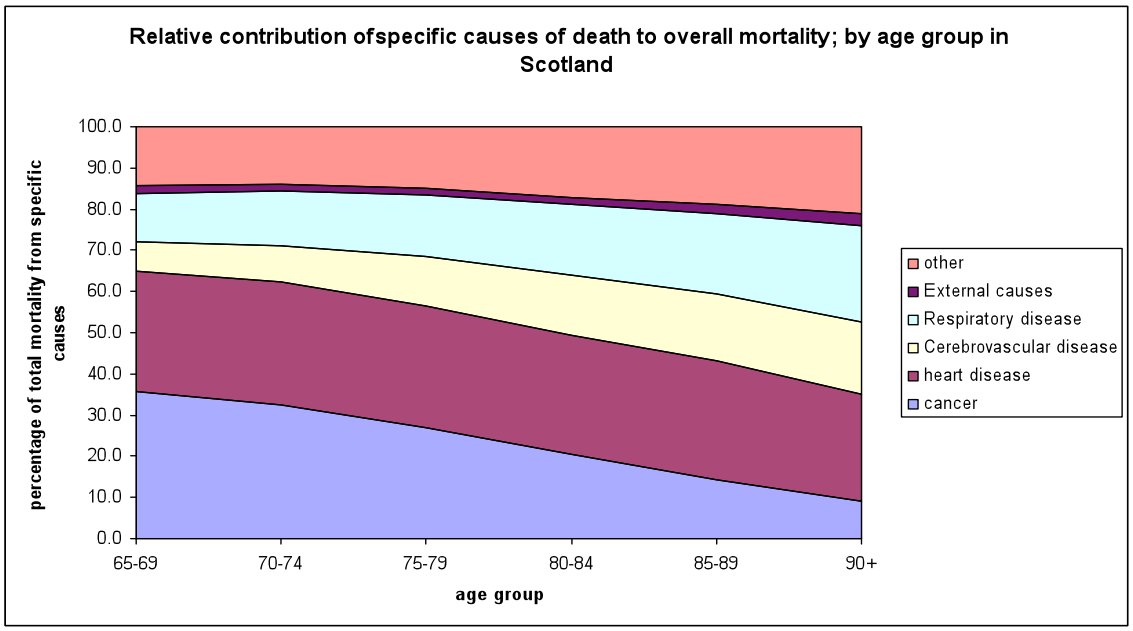
The effect of social circumstances on older people has a huge impact on life expectancy as well as healthy life expectancy, defined as "the number of years a person can expect to live in good health" with the least well off and those living in areas of deprivation having poorer health and a shorter life expectancy. Healthy Life Expectancy was considerably lower in the most deprived areas of Scotland with a healthy life expectancy at birth of only 57.5 years and 61.9 years for males and females compared to 67.9 years and 69 years in males and females respectively for the rest of Scotland in 2009[6].

This demonstrates the impact that socio-economic deprivation has on health status in older people. Living in areas of social deprivation can be detrimental to the lives of older people due to poor public transport and the poor quality of housing. In addition, high crime rates and social isolation, also impact on their health and wellbeing.

Morbidity and mortality

Mortality rates rise with age. The causes of mortality also differ with age. Approximately 80% of deaths in people over 65 years old are attributed to cardiovascular disease, stroke and respiratory disease. Cardiovascular disease is by far the commonest cause of death in the young old (> 65 years old), whilst cancer accounts for relatively few deaths in the very old (>85 years old). (see Figure 1.3).

Figure 1.3. – The relative contribution of specific causes of death to overall mortality; by age group in Scotland[7].



Chronic diseases are more common in older people and are associated with a higher use of primary and secondary care services[7]. The rates of chronic illnesses and disability increase dramatically with age especially in patients over 85 years. Around 60% of people over 85 years report having longstanding limiting chronic illnesses and of these people, approximately 80% report having a disability[8]. Circulatory, respiratory and musculoskeletal disorders account for the largest proportion of illness and disability in older people in Scotland.

Implications of an ageing population on the health service

Many researchers have predicted that an increased ageing population will have huge social, political as well as economic implications especially in the developed world, where an ageing population has been blamed for an increase in Health Care Expenditure (HCE)[9].

In Scotland £4.5 billion was spent on health and social care for people aged 65 years and older from 2006 to 2007. The majority of this was spent on hospitals and care homes with hospital emergency admissions costing £1.4 billion alone[10]. In the face of a rising older population, the total health and social care expenditure in Scotland would need to increase by 78% to £8 billion, if demand for services continues in this pattern.

In contrast to other countries the rise in the number of older people has not meant a rise in healthcare expenditure. In a UK longitudinal study, NHS expenditure was shown to rise between 1985 and 1999. However, elderly patients had decreases in per capita costs compared to the middle age groups. Costs per capita for those individuals over 65 years old in England and Wales showed a rise of only 8% compared to Japan, Canada and Australia where a larger increase per capita costs of 12%, 20% and 56% respectively has been recorded [11]. However, it is important to note that this difference is more likely to account for different health care systems and the way in which funding is divided between the different health care sectors. In the United States Medicare Current Beneficiary Survey, information was gathered from over 12,500 Medicare clients aged over 65 years. The survey showed despite their greater longevity, healthy older people had similar cumulative health care expenditures than

those in poorer health[12]. This suggests that efforts in health promotion and disease prevention may help people to live longer without increasing healthcare costs.

Recent standards proposed by the National Service Framework for Older People in England have encouraged health promotion in older age. Studies have shown that identifying risk factors and implementing strategies to modify biological, social, psychological and environmental risk factors can reduce the onset of disability in later life[13]. This was demonstrated in a randomised controlled trial by Stuck *et al*, where functionally impaired older people had regular multidimensional geriatric assessments. The study showed that early intervention and risk stratification reduced disability, reduced nursing home admissions and also led to reductions in health care costs of $1403 per patient per year[14]. This demonstrates that developing interventions to reduce disability in later life may help reduce the health care costs for older people.

1.2 Decline in physical function in later life

Background

Impairment of functional status has frequently been associated with poorer health outcomes[15]. The Framingham Disability Study, one of the largest population studies to date, suggested a strong relationship between increasing physical disability and age, especially in people aged between 55 to 84 years old[16].

Within the Western World, approximately 13-14% of the total population suffer from a degree of disability which places a huge demand on rehabilitation services[17]. Disability among older people is not a new problem. Statistics from the Office of Population Censuses and Surveys (OPCS) estimated that there were approximately 4.3 million disabled people in the UK over 60 years of age, with functional impairment due to musculoskeletal disorders reported as a common cause of disability in the elderly [18]. Unsurprisingly, the highest disability rates were in institutionalised people. Functional decline is a common yet serious problem in older people which can result in the loss of independence. More importantly, it can directly impact on quality of life[19].

Models of disability and functional impairment

Models of illness are useful when conducting research, designing new interventions or aiding the allocation of resources. In 1980, a model of the disability process was published and the International Classification of Impairments, Disabilities and Handicaps (ICIDH) was subsequently developed by the World Health Organisation (WHO) (see Figure 1.4)[20]. The aim of the model was to allow an understanding of disability. It was thought that a universal classification system for disabilities would

lead to developments in public health and social policies. It also helped clarify definitions of functional impairment.

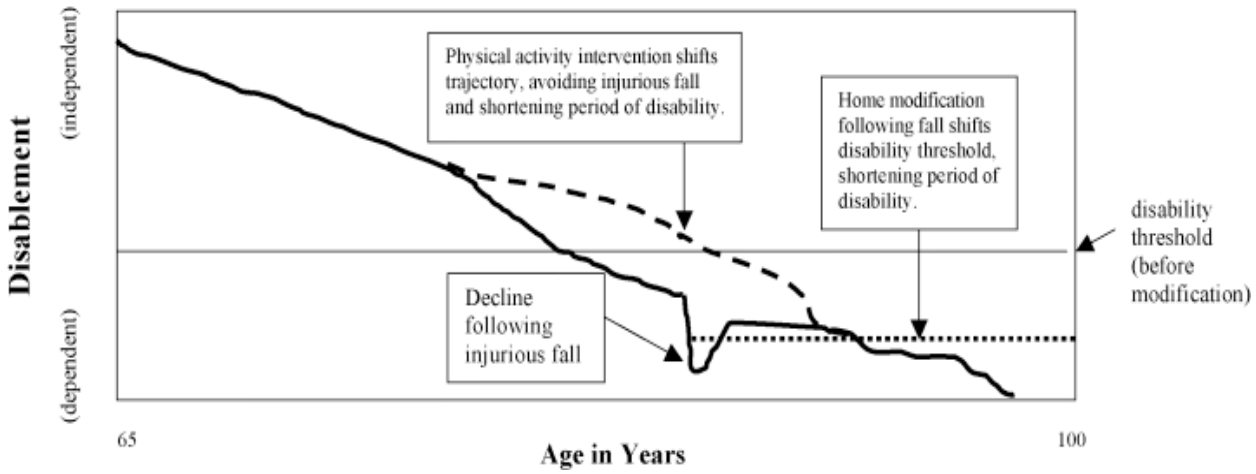
Figure 1.4 – The Disablement Process (adapted from ICHDH, WHO 1980)[20]

Disease Or **Disorder**	→	**Impairment** (functional loss) [organ level]	→	**Functional Limitation** (activity limitation) [personal level]	→	**Disabilty** (social disadvantage) [social level]

The model shows the pathway from pathology of a disease to disability. Both acute and chronic diseases can cause disability, however the onset of disability from chronic disease is more progressive. According to the model, an underlying disease or disorder can lead to dysfunctions or structural abnormalities of specific body systems i.e. musculoskeletal, cardiovascular or neurological system, resulting in 'impairment'. This may lead to 'functional limitation', where an individual is restricted in performing basic physical or mental actions. Limitations in physical function can lead to difficulties performing basic activities of daily living (ADLs) such as personal hygiene, grooming, dressing, feeding, functional transfers, continence and ambulation or difficulties in household management, instrumemental activities of daily living (IADLs), such as shopping and cooking. 'Disability' occurs when an individual loses their ability to carry out daily tasks, preventing them from fulfilling their normal role[20]. History of disability can lead to a vicious cycle of further decline in functional state, flare-up or exacerbations of disease and the involvement of other body systems.

Decline in physical function has been associated with higher rates of falls, loss of independence, institutionalisation and mortality[21]. Freedman et al showed how identifying and managing modifiable risk factors may alter the disablement process. Diseases such as musculosketal disorders or disabling strokes are strongly associated with functional impairment and physical disability. For example, in a patient who has suffered a recent fall resulting in an injury, introducing interventions such as a physical activity program may help promote muscle strength and balance and lead to a reduction in falls risk, therefore shortening the disability period, as shown in Figure 1.5[22]. Other useful interventions may include home modifications such as inserting hand railings and a shower seat may help the individual to regain their normal functioning and also prevent further functional decline and disability. As the majority of older people lie on or around the functional limitation threshold even a small change in their functional state can lead to increased disability and loss of independence. Therefore, identifying and managing risk factors to prevent decline in physical function at an early stage may help to delay the onset of disability and reduced morbidity and mortality in older people.

Figure 1.5 – Interventions which may alter disability trajectory. (taken from Freedman et al)[22].



Risk factors for decline in physical function

Reduced lower extremity muscle mass

Muscle strength is essential for overall physical performance, stamina and balance. Existing evidence has shown the importance of muscle weakness in predicting physical functional decline and future disability[23]. Improving muscle strength through physical training can lead to improvements in physical function. More recently studies have also investigated the influence of muscle mass on physical function. In a study involving frail older community dwelling participants aged 65 years and above, the loss of total lean leg mass (TLM) was an independent predictor of functional impairment, after adjusting for underlying co-morbidities, body weight, body fat, physical activity and bone mineral density[21]. The study also showed that loss of TLM in older people was strongly associated with reduced muscle strength ($p< 0.01$, r=

0.78) as well as physical disability. The study also showed that for every 1Kg increase in muscle mass there was a 53% reduction in functional limitation.

There is growing evidence to suggest that other factors such as muscle composition including fat infiltration and type of muscle fibre could also influence muscle strength and functional performance[24].

Co-morbidities

It has long been established that decline in functional status in older people has been linked to underlying diseases. This has been well represented in the WHO models for disability. It is thought that it is the loss of function at organ level due to an underlying disease process that contributes to loss of function.

A number of studies have assessed the relationship between underlying disease and disability. Several chronic diseases appear to feature more frequently in older people with disability than those without any disability. The Women's Health and Aging Study screened over 3841 community dwelling female participants over 65 years. The study showed that women who were disabled reported more than a four-fold increase in the frequency of stroke, a three fold increase in heart disease and were twice as likely to suffer diabetes or arthritis compared to those who were classified as having no disease[25].

Several studies have tried to link certain chronic diseases which are most likely to cause disability. Patients with conditions such as osteoarthritis, stroke, heart disease and depression, report the highest levels of physical disability[26]. Despite the low

prevalence of stroke, it appears to have a large impact on functional limitations in a wide range of daily tasks.

In reality, older people usually suffer from multiple co-morbidities. In fact, some cross-sectional studies have shown that the number of co-morbidities can be predictive of future disability. Patients with multiple chronic diseases are at increased risk of loss in mobility [27]. This demonstrates the direct impact of co-morbid disease on functional state. This would suggest that providing early medical intervention in both single and multiple co-morbid diseases can minimize the degree of disability.

Depression

As well as affecting general well-being, depression is also thought to contribute to functional decline. A number of studies have observed an association between depression and disability in older people. In hospitalized patients with symptoms of depression, as measured by a Geriatric Depression Scale of >6, patients were more likely to be limited in at least one ADL on discharge and 90 days post discharge[28]. Even after adjusting for other confounding factors such as alcohol intake, cognitive function and chronic conditions, the presence of depression is still a significant predictor of functional decline[29]. This may lead to a spiraling effect as limitations in ADLs are themselves associated with a number of negative health outcomes such as hospitalization and mortality which in turn may cause further physical and psychological decline. The exact mechanism between depression and physical performance is still not clearly understood. It has been postulated that depression may be a pro-dromal syndrome for other medical conditions which precipitate functional

decline. A common feature of depression is apathy, which can lead to a lack of motivation required to maintain physical function.

Slow gait speed

Physical performance tests have been found to be valid predictors of morbidity and mortality as well as disability[30]. Of the available physical performance tests, gait speed has been one of the simplest yet effective tests to perform in a clinical setting. In a large prospective study, participants with a gait speed of less than 1 metre per second (m/s) had a two-fold increased risk of functional impairment of the lower limbs. The study also showed that a slower gait speed was associated with increased mortality (RR=1.64) and hospitalisations within 1 year (RR= 1.48)[31]. This demonstrates the use of gait speed as a valid predictor of functional impairment and mortality in older people[32].

Alcohol

Lifestyle factors including alcohol consumption can influence physical function. La Croix *et al* showed that 'heavy alcohol consumption' in older people, regarded as >28g of alcohol per day, resulted in a 20% increased risk of a decline in mobility in men and a 40% risk of a decline in mobility in women[33]. It is possible that heavy alcohol consumers may be at risk of having falls and fractures as a result of their alcohol intake. Interestingly La Croix also showed that individuals with mild-to-moderate alcohol consumption were more likely to maintain their mobility than people who abstained from alcohol. This was supported by Guralnik *et al*, in a prospective study in older people, where people with moderate alcohol consumption were 2.4 times more likely to have a higher level of physical function compared to those who were

abstinent from alcohol[34]. These benefits may be explained by the fact that moderate alcohol consumption has shown to reduce the risk of cardiovascular heart disease and thus reducing related disability[35].

Obesity

Obesity is growing in prevalence throughout the developed world and is associated with functional limitation and negative effects on quality of life. Increased body mass index (BMI) is closely associated with functional decline. Obesity, defined as a BMI > 80th centile, was associated with a 20% reduction in mobility in men compared to a 40% reduction in women[33]. A low BMI was not significantly associated with mobility decline in either sex. Studies have also shown that older people with a high percentage of body fat are also associated with functional decline and disability[24]. Possible mechanisms for the association between obesity and functional decline include its correlation with increased risk of cardiovascular disease events which independently leads to functional decline[36]. Obesity is also associated with decreased levels of physical activity which, in turn, can lead to de-conditioning and functional limitation. Obesity may lead to increased mechanical stress to joints and hence lead to possible debilitating bone disease such as osteoarthritis. Studies have demonstrated that inflammatory cytokines, including interleukin-6 and tumour necrosis are secreted by adipose tissue explants from obese individuals[37]. This has led to the hypothesis that obesity may represent a low-grade chronic inflammatory state.

Sensory impairment

Many studies have suggested that self-reported poor vision is associated with increased risk of functional decline. There is evidence that patients with severe visual impairment, measured by visual acuity, had a three-fold greater risk of subsequent functional decline[38]. In comparison, the association between hearing loss and functional impairment is less significant, especially when data was controlled for age and other co-morbidities[39]. This may be due to the fact that in most studies hearing loss was self-reported. Visual impairment may lead to an increased risk as well as fear of falling which could influence functional impairment.

Increased frequency in falls

Unintentional injuries, as a result of falls, are still the fifth leading cause of death in older people in the US after cardiovascular disease, stroke, respiratory disorders and cancer[40]. It is thought that 12% of all falls will lead to serious injury, including fractures[41]. In the UK, the incidence rate of falls in people >60 years old is 3.6/100 persons per year, with mortality of people with recurrent falls twice that of people without falls[42]. Whilst a single fall is not significantly associated with an increased risk of future functional disability, recurrent falls are associated with an increase in functional disability and institutionalisation in older people[43;44]. People with recurrent falls have a four-fold increase in disability compared to those without falls[45].Older people who fall are also more likely to experience a fear of falling, which may result in restriction of their ADLs[46].

Cognitive impairment

There is a strong association between progressive cognitive impairment and functional decline. A randomised controlled trial investigating the benefits of a home-based exercise training program in community dwelling frail older people, showed that subjects with poorer cognitive function Mini-Mental State Examination (MMSE) <24 showed the least improvement physical function[47]. This maybe due to the fact that subjects with poorer cognitive function had more difficulties in following instructions and in retaining information and are therefore, less likely to benefit from physical interventions.

Physical inactivity

Physical activity is any bodily movement produced by skeletal muscles which require energy expenditure. It has been well proven that regular physical activity contributes to primary and secondary prevention of a number of chronic diseases as well as reducing overall mortality and premature death[48;49;50].We know that increased physical activity reduces the risk of physical disability. People who engage in regular physical activity more than three times per week are less likely to have a decline in mobility than older people with sedentary lifestyles[51]. The participation in regular physical activity can reduce mobility loss in healthy older people by up to 50% over several years[52].

However, there is controversy as to the type and level of activity required to achieve maximum reduction in the rate of decline in mobility and hence functional decline. A number of studies have shown that only vigorous or high intensity exercise regimens are beneficial in reducing functional decline[53]. However, more recently evidence has shown benefits in low intensity exercise such as walking, gardening and housekeeping,

on physical function, which is a more attractive goal especially in frail older people with multiple co-morbidities[54]. Although there is strong evidence to support physical activity reducing the risk of physical functional decline, most studies have only included people with preserved functional status at baseline. This means that frailer older people have not been well represented. One large European observational study investigated the benefits of moderate physical activity in frail older people ('frailty' defined as problem in performing one or more ADL). It concluded that there was an almost two-fold increase in dying without disability among those who were physically active compared to those who led sedentary lifestyles[55]. This demonstrates the beneficial effects of physical activity in a large proportion of the older population but there is a need for further research into the influence of physical activity in functionally impaired older people.

Functional decline is associated with a number of risk factors. Given our globally ageing population, there is pressing need to identify and evaluate interventions that have the potential to minimise functional impairment and delay the onset of disability in later life.

1.3 Ageing muscle and Sarcopenia

Background

Maintaining muscle function is vital to remain functionally independent. Muscle mass and force reach their peak between the fourth and sixth decades of life and thereafter show a steady decline with age[56]. With this progressive loss of skeletal muscle mass comes a reduction in mobility which, in turn, leads to increased frailty and disability.

Epidemiology of sarcopenia

'Sarcopenia' is a syndrome characterized by the progressive generalised loss of skeletal muscle mass, strength and quality which occurs with age. The word is derived from the Greek word *'sarx'* meaning flesh and *'penia'* meaning loss[57]. Sarcopenia is usually accompanied by physical inactivity, decreased mobility, slow gait and poor physical endurance which are common features in frailty syndrome[58]. Rockwood *et al* described the concept of frailty as "a multidimensional syndrome which involves loss of reserves of energy, physical activity, cognition and health, which give rise to increased vulnerability"[59]. The frailty process involves a cumulative decline in multiple physiological systems in particular there is a decline in the neuromuscular system that may be linked to the age-related development of sarcopenia. This loss of muscle mass has significant implications to a person's functional status as it can cause a reduction in muscle strength and exercise capacity, which are required to perform ADLs. A reduction in muscle mass is also a strong predictor of mortality in later life[60]. As well as the huge burden of the clinical consequences of sarcopenia the economic impact on the health care system is substantial with annual costs attributable to sarcopenia estimated at $18billion in the United States[61]. With an estimated 15% of

people over 65 years old and 50% of people aged over 80 years[62] developing sarcopenia, research into understanding the mechanisms behind this phenomenon and developing approaches to counter the effects of sarcopenia, should be a high priority.

The overall prevalence of sarcopenia in our ageing population remains uncertain due to the lack of consensus on the definition and the variations in measuring age-related changes in skeletal muscle mass and strength. More recently, attempts have been made to refine the definition of sarcopenia. A joint effort was made between the European Society of Clinical Nutrition and Metabolism and the Special Interest Needs Group for geriatric nutrition on 'Cachexia – anorexia and chronic wasting disease'[63]. They suggested that a diagnosis of sarcopenia could be made based on two of the following:

i) A low muscle mass, i.e. a percentage of muscle mass > 2 SD's below the mean, measured in groups of young adults of the same sex, age and ethnic background.

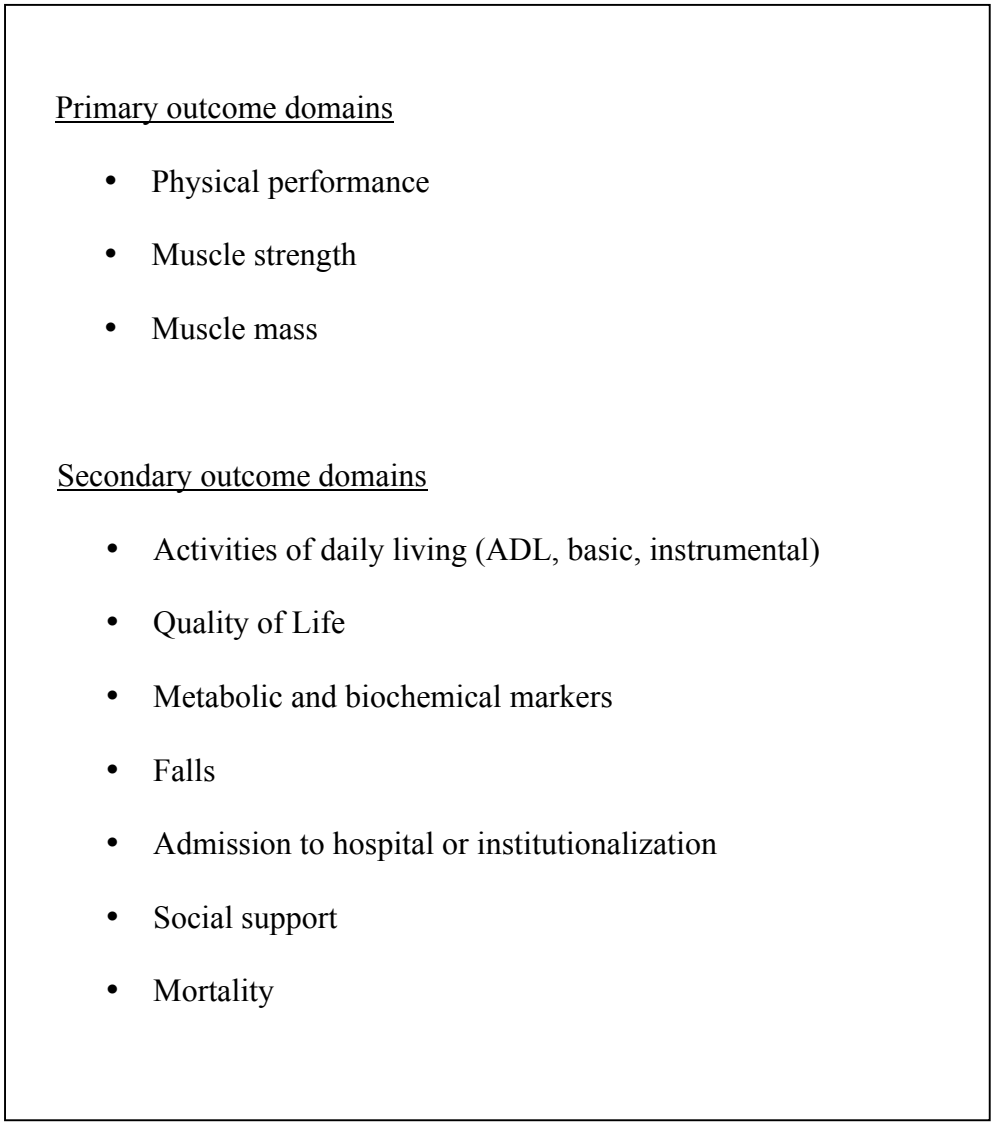
ii) Low gait speed, i.e. a walking speed of <0.8m/s in the 4-metre walk test.

This definition included measures of physical performance while eliminating variance between ethnicity and sex. However, it failed to set age-specific populations. It has been suggested that a T-score system, similar to that used for diagnosing osteoporosis, could be useful to provide reference values for different populations. However, this too requires further refinement.

The European Working Group on Sarcopenia in Older People (EWGSOP) (a steering group involving four European Scientific organizations of Geriatric Medicine and Clinical Nutrition) met in January 2009, to develop an operational definition and diagnostic criteria for sarcopenia for use in research studies and clinical practice[64]. This would help to identify the possible mechanisms of sarcopenia, thus enabling the

development of effective treatments to prevent the loss of independence and to limit disability in older people. With the knowledge that sarcopenia is a dynamic process, the EWGSOP recommended that sarcopenia should be diagnosed *"in the presence of low muscle mass and low muscle function (strength or performance)"*[64]. Until recently, a major stumbling block for developing potential treatments for sarcopenia was the absence of standardized primary and secondary outcomes for clinical trials. However, the EWGSOP recommended primary outcome measures based on muscle mass, muscle strength and physical performance as shown in Figure 1.6.

Figure 1.6 Primary and secondary outcome domains for interventional trials in sarcopenia (adapted from the EWGSOP 2009[64])



Diagnosis of sarcopenia

Until recently, the main difficulties in diagnosing sarcopenia was the lack of consensus in its definition in addition to difficulties in measuring changes in muscle mass and function over time in older people. Although sarcopenia is considered a dynamic process, incorporating both changes in muscle mass and function, many observational studies have concentrated on measuring muscle mass and in particular fat-free mass, but failed to measure this alongside physical function.

Measuring techniques for sarcopenia.

Muscle mass

Previous cadaveric studies have shown that computerised tomography (CT) and magnetic resonance imaging (MRI) provide reliable measurements of skeletal mass. However, routine use of such technologies could lead to an escalation of cost as well as risks involves with exposure to radiation in CT scanning. More recently, Dual Energy X-ray absorptiometry (DXA) has become a possible alternative for skeletal muscle estimations as it measures both fat mass as well as bone mass and is accurate at measuring appendicular muscle mass. DXA scanning correlates with measurements of skeletal mass achieved via MRI scanning[65]. However, the main limitation is the ability of the DXA scan to distinguish water retention and muscle fat infiltration. Studies have shown that muscle fat infiltration can over-estimate skeletal muscle mass by 1-8% and therefore may wrongly identify some individuals of the population as sarcopenic[66].

Other approaches of measuring body composition within the past twenty years include the use of bioelectrical impedance analysis (BIA). This method is a quick and non-invasive approach for assessing body composition by measuring tissue conductivity

and is a relatively inexpensive alternative measuring technique for large population studies. Results from BIA have also shown to correlate with MRI measures of muscle mass[67]. Using BIA scanning, the New Mexico Elder Study calculated the skeletal mass by using an anthropometric equation for predicting appendicular skeletal muscle (ASM) as measured in all four limbs. In this study, sarcopenia was defined as ASM/height^2 (Kg/m^2) of less than two standard deviations below the mean of a young reference group. The prevalence of sarcopenia ranged from 13-24% in people aged 65-70 years and the prevalence rose to 50 % in those aged over 80 years old. The study also showed that the prevalence was higher in men aged over 75 years (58%) compared to women in the same age category (45%). This was due to the fact that older men have a higher proportion of lean muscle mass than women[68]. It was later recognised that the results using the BIA method may be grossly overestimated and a subsequent study was done using DXA scanning. The results from the DXA scanning method showed a much lower prevalence with 16% of women and 29% of men over 80 years being classified as sarcopenic[69]. A cross-sectional survey in older community dwelling people in Minnesota defined sarcopenia using total rather than skeletal body muscle mass. The population prevalence was much lower with 6 to 15% of participants with sarcopenia[70]. It is worth noting that using total body mass measurements may introduce errors as a result of a potential rise in water and fat content in ageing muscle. As the study involved a community based population, participants may have been slightly healthier with fewer underlying co-morbidities.

Anthropometric measures, including mid-upper arm circumference (MUAC) and triceps skin fold (TSF) thickness, have been used to calculate muscle mass in many research studies. Rolland *et al* showed that calf circumference positively correlated

with muscle mass and that a calf-circumference of <31cms was associated with increased disability. However, the main disadvantage of using anthropometric measures is that they are subject to errors due a loss of skin elasticity in older people and in cases of 'sarcopenia obesity', where there is a loss of lean muscle mass but an increase in muscle fat deposition[71] . Therefore, anthropometric measures should not be used as a screening tool for sarcopenia.

A summary of current measuring techniques used including their limitations is shown in Table 1.1.

Table 1.1- Measuring techniques for sarcopenia (taken from Burton et al.)

Measuring techniques	Measurements	Comments
Muscle size		
CT Scan	Muscle cross-sectional area	Radiation exposure, expensive
MRI Scan	Muscle cross-sectional area	Expensive, availability of MRI
BIA	Tissue conductivity	? reliability
Muscle circumferences	Mid arm and calf circumference	Measurements effected by subcutaneous fat
DXA scan	Total skeletal muscle mass	Reliable, low radiation exposure
Physical performance		
SPPB	Lower extremity function	Validated tool for older people

Abbreviations: CT, computed tomography; MRI, magnetic resonance imaging; BIA, bioelectric impedence analysis; DXA, dual energy X-ray absorptiometry; SPPB, Short Physical Performance Battery.

Muscle strength

The main effect of loss of muscle mass is loss of strength. Low muscle mass is associated with increased mortality. Although complex measures of power and torque are available, one of the simplest measures of muscle strength is by using hand-held dynamometers that measure hand grip and quadriceps muscle strength. These have been used frequently in research. Hand grip strength is not only simple to use in a clinical setting but it has good reproducibility. Also, it also correlates to lower limb muscle strength[72]. A handgrip strength of <30Kg in men and <20Kg in women has

been associated with increased mobility limitation and disabilty[73]. One of the main disadvantages of using hand-held dynamometers is that patients with osteoarthritis and other co-morbidities may be limited in performing the technique due to pain. This may lead to under-estimation of muscle strength in some older people.

Physical performance

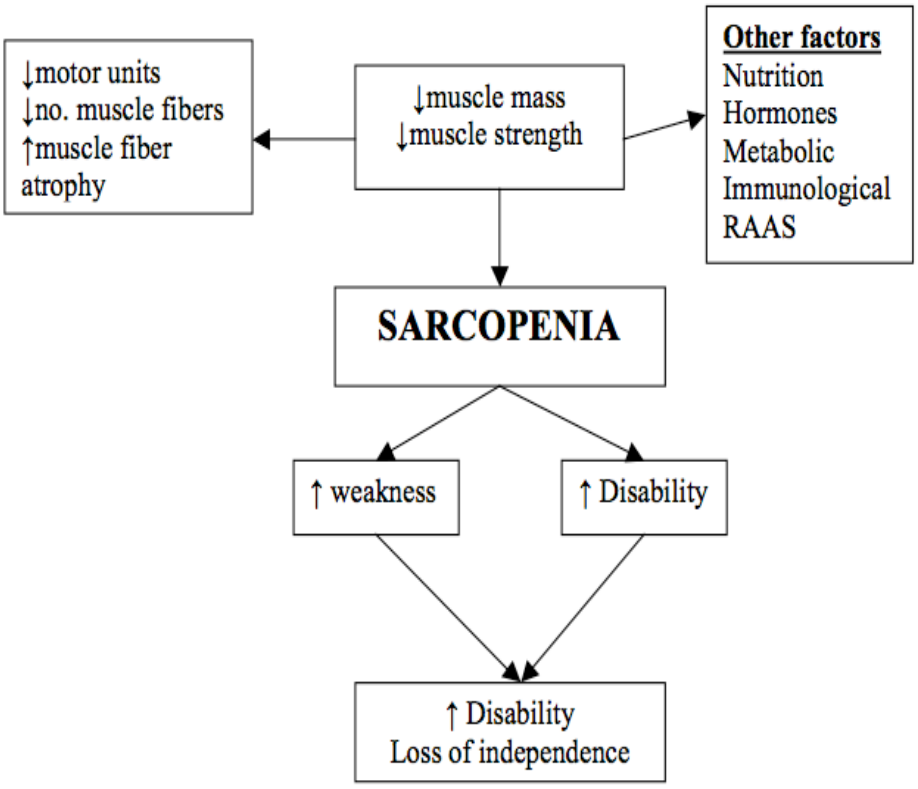
Physical performance measures can complement measures of muscle mass and strength in the diagnosis of sarcopenia. The Short Physical Performance Battery (SPPB) assesses muscle function and strength using measures which are relate to daily living activities. The assessment involves balance tests, a 4-metre walk test and a timed chair rise which gives an overall score and can be performed in a clinical setting. It can also predict future disability and therefore, may be useful at identifying people at the preclinical stage of sarcopenia, who may benefit from interventions[74].

Gait speed has shown to be an independent measure of physical performance and muscle strength and is a good predictor of disability[32]. Buchner *et al* showed that in frailer older adults there was a curvilinear relationship between walking speed and leg strength, showing that small changes in physiological capacity (muscle strength) may have substantial effects on physical performance (gait speed)[75].

Aetiology of sarcopenia

An understanding of the mechanism involved in the development of sarcopenia is essential in order to develop interventions. Research is still ongoing but, as yet, no primary cause has been identified in the development of sarcopenia. It appears that there are multiple risk factors and mechanisms which contribute to the development of sarcopenia as shown in Figure 1.7.

Figure 1.7 – Mechanism of sarcopenia



Loss of neuro-muscular function

Disturbances in neuro-muscular function increase with age. Ageing is associated with increasing muscle weakness and slowing of the muscle, resulting in a loss in power generating capacity. The combination of disturbed motor control and loss of muscle mass has a huge impact in physical functioning as well as quality of life in older people.

In ageing muscle, loss of alpha motor neuron axons appears to be maintained until the sixth decade of life, after which there appears to be a rapid decline[76]. Histological analysis, from muscle biopsy sampling, has given a great insight into age-related muscle atrophy. The loss of the alpha motor neurons appears to affect the longer axons in the lower extremities first before the upper limbs[77].

Muscle contraction is initiated and maintained by the successive recruitment of motor units. A motor unit encompasses an alpha motor unit and the muscle fibres which it innervates. One of the major contributing factors, to age-related loss of muscle force and power, is through motor unit re-modelling. During this process muscle fibres are progressively de-nervated and either irreversibly lost or re-innervated by the sprouting of the remaining motor neurons.

Structural and functional changes occur with age in the skeletal muscle. The co-expression of the two myosin heavy chain (MHC) isoforms determines the muscle fibre morphology. When MHC isoform 1 is predominant, it favours type I muscle fibres. Type I muscle fibres appear to be slow-twitch fibres which are fatigue resistant. They have a greater oxidative capacity and are associated with endurance. On the other

hand, when MHC isoform IIa and IIb are predominant, it favours type IIa and type IIb muscle fibres which, in contrast, are fast-twitch fibres that fatigue easily and have a high glycolytic capacity. Type II muscle fibres are associated with muscle power[78].

Both animal and human studies have shown a preferential loss of fast twitch type II muscle fibres with increasing age. The extent of muscle fibre loss between muscle fibre type in ageing muscle appears to vary between study, with some studies showing a decrease in type II fibres while others have found no difference in numbers between types[79]. Despite this, the evidence suggests that only 10-15% of motor neuron loss occurs with advancing age[80]. This demonstrates that muscle fibre loss alone is not sufficient to impact on functional loss in ageing skeletal muscle; rather it is the complex interaction between many factors including changes to neuromuscular transmission, muscle architecture, muscle fibre composition and muscle metabolism.

Several studies have reported skeletal muscle de-nervation and re-innervation, motor unit remodelling or loss, both in humans and rodents. This remodelling leads to changes in the fibre type composition. During the ageing process, remodelling of the motor units appears to involve de-nervation of the fast muscle fibres with re-innervation by axonal sprouting from the slow muscle fibres. This could be a major contributing factor for sarcopenia[81]. When the rate of de-nervation is higher than the rate of re-innervations, a proportion of the muscle fibres become atrophic leading to functional impairment with ageing.

Satellite cells

The effect of ageing on human muscle fibres is unique. Satellite cells are key cells in the regeneration and growth of muscle fibres. They are effectively myogenic stem cells, located just outside the muscle fibre sarcolemma but beneath the basement lamina. They are quiescent cells that become activated by stimulation such as in muscle fibre injury. When injury occurs, they start replicating and are responsible for the repair of injured muscle fibres, differentiation of new muscle fibres and generation of new satellite cells.

It is postulated that, during the ageing process, there is a reduction in the number of satellite cells and their capacity to differentiate muscle fibres may be altered as there is a greater reduction in type II rather than type I muscle fibres[82]. Despite the reduction in satellite cells, the remaining intact satellite cells must be sufficient to generate some muscle repair in ageing muscle[83].

Alterations in calcium homeostasis

The ageing of skeletal muscle is also linked to age-related changes in calcium homeostasis. These alterations in calcium during ageing have shown to involve changes in the excitation-contraction coupling (EC) process, changes in transient Calcium (Ca^{2+}) levels, alterations in the chloride and potassium channels as well as slowing down of contraction times.

Skeletal muscle contraction is initiated from action potentials generated by the motor neuron and conducted via the axons, which causes the release of acetylcholine (Ach) at the motor end plate. Ach then binds to the nicotinic Ach receptors, which in turn

causes an increase in the conduction of sodium and potassium from the end plate membrane. End plate potentials at the muscle membrane lead to generation of action potentials and conduction to the sarcolemma T-tubules. This then leads to intracellular elevation in calcium concentration which precedes muscle contraction.

In ageing, the muscle fibres exhibit a significant decrease in sarcoplasmic calcium concentrations as a result of a reduction in L-type Ca^{2+} channel charge movement. There also appears to be a decline in dihydropyridine receptors (DHPR). DHPRs act as voltage gated sensors located in the sarcolemma T-tubules which when activated provoke Ca^{2+} release from the sarcoplasmic reticulum. This release of Ca^{2+} from the SR (sarcoplasmic reticulum) is also aided by the modulation of ryanodine-sensitive calcium channels and by sarcoplasmic reticulum protein calsequestrin. The ryanodine receptors act to release Ca^{2+} during the excitation and coupling process[19]. The consequence of a reduction in the number and function of these receptors and proteins causes a reduction in the intracellular Ca^{2+} movement and therefore a reduction in force which is common in ageing muscle.

Reductions in peak intracellular Ca^{2+} levels appear to affect slow-twitch muscle fibres more than fast twitch muscle fibres in older rats, resulting in a prolonged time to peak force and a longer contraction duration[84]. The uncoupling of machinery for the excitation contraction process is a key factor in age dependent decline in the capacity of the muscle to generate force. In experimental rodent studies, de-nervation causes a decrease in expression of DHPR receptors and therefore may alter the excitation-contraction coupling and muscle contraction. This demonstrates the importance of alterations in Ca^{2+} homeostasis on ageing skeletal muscle[85].

Pro-inflammatory cytokines

Along with a decrease in anabolic stimuli, ageing appears to produce increased catabolic stimuli. There is some evidence that ageing contributes to a rise in pro-inflammatory cytokines.

Cachexia of old age is thought to be "a cytokine-associated wasting of protein and energy stores due to an underlying disease process"[86]. It is thought to trigger an inflammatory response mediated through cellular injury or through activation of the immune system, which produces an acute inflammatory response. This process has shown to produce a sharp rise in inflammatory cytokines interleukin 1 (IL-1), interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF-α). These cytokines have a direct negative effect on muscle mass and reduce lean muscle mass. High levels of cytokines are associated with cachexia which is a common occurrence in many chronic medical conditions affecting the elderly, such as heart failure, COPD and cancer. Pro-inflammatory cytokines have also been found in otherwise healthy older people. Therefore, the ageing process itself has been associated with a mild inflammatory response and increased catabolic stimuli[87].

A number of studies have suggested a link between cytokine production, signalling and sarcopenia. Cytokines such as TNF-α and IL-6 appear to play an important role in age-related inflammatory responses and may also play a role in the development of sarcopenia. Elevated levels of TNF-α in aged muscle are thought to stimulate muscle loss through the activation of apoptosis pathway and impair inflammatory response to injury [88]. When IL-6 is expressed at low levels it appears to act as a muscle growth factor but, in contrast, when expressed at higher levels it appears to cause muscle

wasting [89]. In addition, higher levels of IL-6 in community dwelling older people are associated with increased functional impairment and physical disability in old age[90]. Despite this, cytokine levels can often be inconsistent and therefore it still remains unclear as to whether the rise is solely due to the ageing process or whether there is indeed underlying disease. It is also worth noting that cytokines such as IL-6 can be stimulated by other factors such as smoking, obesity and stress and this should be taken in to consideration when interpreting the data [91].

In experimental studies with rats, the administration of IL-6 or TNF-α increases skeletal muscle protein breakdown, decreases the rate of protein synthesis and caused muscle wasting[92]. In cross-sectional studies in humans, higher levels of IL-6 and TNF-α have been associated with lower muscle mass and strength[93]. The Longitudinal Ageing Study of Amsterdam showed that high levels of IL-6 and CRP were associated with an increased loss in muscle strength[94]. In addition, the study demonstrated that the presence of high levels of anti-chymotrypsin (ACT), a serum protease inhibitor which regulates the activity of proteolytic enzymes during inflammation, which reduced the risk of muscle strength loss and therefore protected muscle tissue breakdown. However, in the presence of high levels of IL-6 and ACT there was increased muscle strength loss indicating that IL-6 inhibits the protective role of ACT. However, further research is required to support this suggestion.

Mitochondrial dysfunction

There is increasing evidence now that muscle mitochondrial dysfunction occurs with age in both rats and humans. The changes which are thought to occur include: 1) a reduction in the copy numbers of mitochondrial DNA; 2) a reduction in the concentration of mRNA in genes encoding for muscle mitochondrial proteins; 3) a reduction in the rate of mitochondrial protein synthesis; 4) a reduction in muscle mitochondrial oxidative enzyme activities and 5) a reduction in ATP production[95].

At a cellular level mitochondria are the main producers of energy for skeletal muscles in the form of adenosine triphosphate (ATP) which is produced via the oxidative phosphorylation of adenosine diphosphate and is therefore crucial in the generation of muscle contraction. Mitochondria also act as a key regulator centre of programmed cell death (apoptosis). During ageing there is an acceleration in apoptosis of irreplaceable post-mitotic cells such as neurons, skeletal muscle fibres and cardiac myocytes which leads to loss of function. It is therefore postulated that increased apoptosis induced by age-related mitochondrial dysfunction plays an important role in sarcopenia.

Apoptosis is essential for numerous biological processes including embryogenesis, tissue homeostasis and cell turnover. It is activated via specific cell signalling pathways which in turn causes the release of ‘caspases’ leading to the ‘caspase cascade’ which acts as the executioner of enzymes in cell death[96]. Mitochondria induce apoptosis through a caspase-dependent or caspase-independent pathway. In the caspase-dependent pathway stimulation and activation of initiator caspases (caspases-8, -9 and -12) leads to activation of effector caspases (caspases-3,-6,and -7) which cause cell degradation. Experimental studies analyzing the gastrocnemius muscle of

old rodents have shown a rise in capsase-3 levels which may indicate a possible link between stimulation of a caspase-dependent pathway in mitochondria and a rise apoptosis with age. Mitochondria may also induce apoptosis through a caspase independent pathway by the release of apoptosis inducing factor and endo-nuclease G which are responsible for DNA fragmentation[97]. It has also been reported that mitochondria interact with the endoplasmic reticulum (ER) and disrupt calcium metabolism and thereby disrupt E-C coupling.

The mitochondrion is the main site for production of reactive oxygen species (ROS). Due to its proximity to the electron transport chain (ETC), mitochondrial DNA is therefore very susceptible to oxidative damage by free radicals[98]. Oxidative stress may be involved in ageing by enhancing mitochondrial DNA mutations and deletions. As a result there is a reduction in the process of oxidative phosphorylation and thus decreased mitochondrial ATP production.

Experimental studies have shown that muscle biopsies of the vastis lateralis in a wide age range of healthy volunteers have an abundance of muscle mitochondrial protein and mDNA and mRNA. However, the muscle mitochondrial protein and mDNA and mRNA declined at a rate of 8% per decade. This is thought to occur as a consequence of an increase in oxidative damage with age[99]. This theory is supported by experimental studies conducted in rodents which showed higher mitochondrial DNA oxidation in older rats[100]. In particular, there was a 5% decline in citrate synthase which is a key oxidative enzyme required for ATP production as well as a decline in VO_2. This supports the theory that age is associated with a reduction in muscle mitochondrial DNA and increased DNA oxidation which in turn reduces mitochondrial

gene transcription and proteins and reduced muscle ATP production. As a result these mitochondrial changes may collectively lead to lower physical function in older people.

We know that physical inactivity contributes to sarcopenia[101]. More recently a link was found between mitochondrial function and physical activity. It appears that older adults may benefit from physical activity through exercise induced adaptation in the cellular antioxidant defence system. Aerobic exercise has been linked to increased ETC activity, mitochondrial biogenesis and reduced oxidative damage[102]. This demonstrates that mitochondrial dysfunction is involved in the ageing process and the potentially beneficial effects that physical activity may have in preventing sarcopenia.

Hormonal changes

Hormonal changes appear during the ageing process. Levels of growth hormone (GH), insulin-like growth factor (IGF-1) and androgens, which help regulate growth and development of skeletal muscle, appear to decrease with age.

Growth hormone (GH) is required for maintenance of muscle and bone. GH exerts most of its anabolic actions through insulin-like growth factor 1 (IGF-1) which is synthesized in the liver for systemic release. IGF-1 helps improve muscle function by increasing production of muscle satellite cells as well as stimulating production of muscle contractile proteins[103]. Not only do GH and IGF-1 levels decline with age, the amplitude and frequency of pulsatile GH release is also significantly reduced[104].

The menopause is linked to reduced concentrations of circulating oestradiol in middle aged and older women. There appears to be impaired muscle performance during the

postmenopausal period when ovarian hormone production has decreased[105]. It seems obvious to hypothesize that oestrogen may play a role in sarcopenia in older women. Testosterone is secreted by the Leydig cells in men and ovarian thecal cells in women[106]. Testosterone appears to increase muscle mass and increase muscle protein synthesis[107]. It also increases the number of satellite cells in both animals and humans which are essential for muscle cell function[108].

A substantial number of older men are hypogonadal. Hypogonadism has been defined as a total testosterone concentration of <9.26nmol/l (2SD below the mean for healthy young men). As a result approximately 20% of men >60 years and 50% men > 80 years are categorized as hypogonadal[109]. Circulating testosterone is highly bound to sex hormone binding globulin (SHBG) and as SHBG increases with age the total amount of bioavailable testosterone decreases. This phenomenon has been termed the 'male menopause' or 'andropause' in older men. Testosterone decreases gradually at a rate of 1-2% per year in males from the age of 30 years-old[110]. The overall reduction of testosterone is associated with loss of muscle strength, muscle mass, a reduction in bone mineral density and increased risk of fracture risk following falls[111;112].

Potential interventions for sarcopenia

Exercise and physical activity

Physical activity refers to the body movement that is produced by skeletal muscle contractions and that increases energy expenditure[113]. Evidence has shown that older adults who are less physically active are more likely to have lower skeletal muscle mass and strength and are at increased risk of developing sarcopenia[114].

Exercise is a form of physical activity that is structured, repetitive and is performed mainly to achieve improvement in health or fitness. Although exercise is a form of physical activity not all physical activity is exercise. In aerobic exercise the larger muscles in the body move in a rhythmic manner for a prolonged period of time. Whereas resistance exercise (muscle-strengthening exercises) involves muscles working hard against an applied force or weight such as in weight-lifting. Both aerobic and resistance type exercise training have shown to improve the rate of decline in muscle mass and strength with age[115].

Aerobic activity (swimming, running and walking) has long been linked to improvements in cardiovascular fitness and endurance capacity. Although aerobic exercise is less likely to contribute to muscle hypertrophy, it can increase the cross-sectional area of muscle fibres[116]. Mitochondrial volume and enzyme activity increase after aerobic exercise demonstrating that muscle protein synthesis and muscle quality improve irrespective of age[117]. Aerobic exercise can also reduce body fat including intramuscular fat which in turn improves the functional role of muscle relative to body weight[118]. In contrast to aerobic exercise training, resistance exercise training appears to have a larger effect on augmenting muscle mass and strength and attenuates the development of sarcopenia[119;120]. Improvements in muscle strength can be achieved

with as little as one resistance exercise training session per week[121]. Frontera *et al* demonstrated improvements in muscle cross-sectional area (CSA) by 11% as well as improvement in muscle strength (>100%) after a 12 week period of high intensity resistance exercise training in older men[122]. Similar improvements were seen in muscle strength even in the people >90 years with as little as 10-12 weeks of training[123]. Muscle hypertrophy occurs when muscle protein synthesis outweighs protein breakdown. Older people performing resistance exercise show a marked increase in skeletal muscle protein synthesis without an increase in whole body muscle breakdown. Resistance training in older people increases both mixed-muscle protein synthesis and specific MHC synthesis to the same levels as younger adults[124]. Evidence points to increases in size of both Type 1 and Type II muscle fibres which could explain the improvements in muscle strength and endurance[124]. More recently, it has been reported that when using moderate levels of resistance exercise training, improvements in muscle strength and size in healthy older people were comparable to muscle strength seen in younger individuals. Roth *et al* demonstrated that six months of whole body resistance training in older people (65-75 years) produced gains in muscle cross sectional area similar to those achieved in younger individuals aged 20-30 years[125].

Progressive Resistance Training (PRT) is the most commonly used resistance therapy used in older people. A Cochrane review of 121 randomized controlled trials of PRT in older people showed that doing PRT two to three times per week improved physical function, gait speed, timed get-up and go, climbing stairs and balance and more importantly had a significant effect on muscle strength especially in the high intensity training groups[126].

The majority of studies have shown that resistance exercise training must be carried out at a high intensity in order to show substantial improvements in muscle strength[122;125]. In contrast, in a 26 week study in older healthy adults at both low and high intensity resistance exercise training programs found only a modest improvement in thigh muscle strength in the high intensity resistance exercise training group[127].

Resistance exercise training appears to be relatively safe to perform even in participants with multiple co-morbidities and can help in prevention of falls.[128] It increases muscle cross-sectional muscle area as well as type II (fast twitch) muscle fibres, which leads to overall improvement in muscle power and the ability to improve physical functioning. As a result, this can lead to enhanced ability to perform ADLs, preventing functional decline and disability. Even in very old residents of nursing homes, resistance exercise training showed substantial improvements in muscle fibre cross-sectional area (3-9%), muscle strength (>100%) as well as improvements in physical performance such as gait speed and stair climbing[129;126]. However, participation in regular exercise training requires motivation by the individual which may be difficult for some older individuals. Therefore non-exercise interventions may offer a useful alternative.

Nutrition

Many older adults do not consume sufficient amounts of dietary protein which leads to a reduction in lean body mass and increased functional impairment[130]. The current recommended dietary allowance (RDA) of protein is 0.8g per Kg per day, almost 40% of people >70 years, does not meet this[131]. A low protein diet below the RDA leads to a significant decline in muscle strength and muscle mass in older women[132]. However, even older people who take the recommended RDA for protein continue to have a negative nitrogen balance and may require a diet containing higher protein content than the RDA to maintain their skeletal muscle[133]. Protein and energy supplementation may increase muscle strength even in very old people in the short term but studies have found no definite functional benefit from nutritional supplementation[134;135,136]. Although older people who exercise have increased protein requirements, studies investigating whether nutritional supplementation in combination with resistance training can augment muscle strength gains in older people, have yielded inconsistent results. A randomized controlled trial in nursing home residents undergoing resistance training over 10 weeks, found that an additional 360 calorie supplement increased leg muscle strength[129]. The combination of protein supplementation with a 12 week resistance training program, increased muscle mass but not muscle strength[137]. Many of oral nutritional supplements used in clinical trials are milk based drinks. A randomised controlled trial involving nutritional supplementation in older people post hospital discharge, showed that 73% of eligible participants refused to enter the study as they disliked the milky consistency of the nutritional drinks[135]. This may lead to problems with adherence to nutritional supplementation.

Testosterone

Evidence to support testosterone supplementation is variable. Gruenewald *et al* analyzed twenty-nine randomized controlled trials investigating the effects of testosterone replacement in older men. Some studies found an increase in lean body mass and hand grip strength but no effect on knee extension and flexion strength[138]. Other studies have shown up to 25% increase in leg strength in as little as 4 weeks of therapy[139]. Some studies have found no increase in muscle strength or function but an improvement in lean body mass[140]. Testosterone supplementation has been shown to increase the size of the prostate gland in men[141]. This could be detrimental to older men in whom the prevalence of early-stage prostate cancer is already high[142]. The Baltimore Longitudinal Study on Aging involving 781 men showed a positive correlation between prostate cancer and the blood concentration of free testosterone levels. The likelihood of acquiring a high risk prostate cancer in men >65 years doubled for every 0.1unit increase in free testosterone[143]. This along with other potential side effects of testosterone therapy like fluid retention, gynaecomastia, polycythaemia and sleep apnoea limit it's potential use as an intervention for sarcopenia[141].

Oestrogens

The effect of hormone replacement therapy (HRT) in women is controversial. HRT may attenuate the loss of muscle mass which occurs in the perimenopausal period[144]. Oestrogen replacement therapy has only modest benefits on muscle composition and this may not translate to improved physical functioning[145]. HRT combined with resistance training may have a role in improving lower extremity function. However

more evidence is needed[146]. HRT has been implicated as a risk factor for breast cancer and is therefore not recommended for sarcopenia[147].

Growth hormones

Despite a number of studies which have assessed the administration of GH supplementation, there is still an ongoing debate as to the use of GH supplementation on muscle mass, strength and physical performance. The strongest evidence for the use of GH supplementation appears to be in states of reduced GH secretion. In younger GH deficient adults, GH supplementation for 3 years increased thigh muscle mass, strength and improved exercise capacity[148]. However, in healthy non-GH deficient older people results are more controversial. Some studies have shown an increase in muscle mass but no improvement in muscle strength whereas others have shown an increase in both muscle mass and strength after administration of GH supplementation[149-151]. The failure of exogenous GH to mimic the pulsatile pattern of normal GH secretion has been blamed for the negative results. Alternative potential hormonal interventions include the use of growth hormone releasing hormone (GHRH) which was found to have only a small improvement in muscle strength in older men[152].

Muscle strength increases as a result of resistance exercise training in older adults[119;122]. It was hypothesized that the combination of GH replacement and exercise training may have a synergistic effect on muscle function in older people. However, results proved disappointing and the addition of GH supplementation does not augment the improvements in skeletal muscle brought about by exercise alone[153]. Low GH levels alone may not be responsible for the levelling off of muscle strength seen in older exercising people and that other pathways may be involved.

As it currently stands, the evidence for the use of GH supplementation to counter the effects of sarcopenia in older people is weak. Moreover the majority of trials involving growth hormone replacement therapy in older people have reported a high incidence of side effects including: increased fluid retention, gynaecomastia, orthostatic hypotension and carpel tunnel syndrome[154].

Creatine

Creatine plays an important role in protein metabolism and cellular metabolism. It has been hypothesized that creatine increases the expression of myogenic transcription factors such as myogenin which increases muscle mass and strength[155]. Creatine supplementation increases muscle phosphocreatine levels leading to a decrease in muscle relaxation time[156]. This may increase the ability to perform high-intensity exercise as well as enhance muscle protein synthesis, lean skeletal muscle mass and strength during periods of high intensity training.

To date, several studies on creatine supplementation have shown increased muscle strength and power in both younger men and women but few studies have looked at the effect of creatine supplementation in older people. Some studies have reported no effect of creatine supplementation on muscle strength or function[157]. However, others have reported increments in muscle mass and increased muscle power without adverse effects[158]. There is controversy over whether creatine supplementation increases the benefits of resistance training alone in older people. Some studies have found no added benefit of creatine supplementation to resistance exercise training while other studies

have found a small increase in lean tissue mass with no residual benefit once training stopped[159;160].

Creatine is a natural ingredient of food and the main source is from meat products with an average daily intake of 1g per day. However, creatine supplementation may increase the risk of interstitial nephritis highlighting the need for particular caution about its use in older people[161]. Creatine is not currently recommended for sarcopenia.

Myostatin

Myostatin is a natural inhibitor of growth factor. It was initially discovered when mutations of the myostatin gene was found to correlate with exaggerated muscle hypertrophy[162]. The myostatin gene appears in skeletal muscle cells and functions as a negative regulator of muscle growth, antagonism of which increases satellite cell proliferation[163]. In animal models, it appears that over expression of myostatin induces extensive muscle loss[164]. Polymorphisms of the myostatin gene in humans correlated with measure of muscle mass, strength and physical performance[165].

Agents that target the myostatin pathway may be useful in increasing muscle mass and therefore play a vital role in muscle wasting disorders as well as sarcopenia of old age. Phase II trials have been carried out in muscular dystrophy and initial results have shown that MYO-29 a recombinant antibody to myostatin, had good safety and tolerability profile[166]. Another potential therapeutic approach currently in development is a soluble activin type IIB receptor that binds to the myostatin therefore reducing its availability. Initial results in mice have shown an increase in muscle weight larger than those achieved with myostatin inhibitors[167].

Inhibition of myostatin with follistatin (myostatin antagonist) may have potential therapeutic benefits in the treatment of sarcopenia. Although myostatin deficiency increased muscle mass in mice it impaired the structure and function of the muscle tendons thereby making the tendons smaller, stiffer and more brittle[168]. Older people who are already at increased risk of contraction induced injury may find it more difficult to sustain regular exercise. Further studies are required.

Vitamin D

Vitamin D levels decline with age and cutaneous vitamin D levels are up to four times lower in older compared to younger individuals[169]. It is known that vitamin D plays an important role in bone and muscle metabolism. Several mechanisms have been suggested for the role of vitamin D in muscle function. Vitamin D binding to the vitamin D receptor found in skeletal muscle promotes muscle protein synthesis and enhances calcium uptake across the cell membrane[170]. Low vitamin D levels result in atrophy predominantly of the type II (fast twitch) muscle fibres in common with sarcopenia[171]. In older people, low vitamin D levels may produce functional problems including proximal muscle weakness, difficultly rising from a chair, difficulties in ascending stairs and axial balance problems[172].

The evidence for a benefit in physical performance with supplementation of vitamin D is controversial. Some studies have shown an improvement in muscle strength with intermittent dosing and others have shown small gains in lower extremity strength and less body sway with daily dosing[173]. This improvement has been hypothesized as the

mechanism behind a fall reduction of 23%-53% in older nursing or residential home residents given vitamin D in addition to a reduction in fractures[174;175].

Conversely, other studies have found no benefits on physical function, falls risk or quality of life with vitamin D supplementation in vitamin D deficient people[176-178]. The difference in findings between studies may in part be attributed to differences in the dose of vitamin D used with better outcomes seen when higher doses are used. It has also been suggested that there is a gender difference in outcomes with women standing to gain more from supplementation[174]. Variations between study populations may also affect outcomes with the biggest improvements in muscle function and physical performance seen in institutionalized older people.

The prevalence of vitamin D insufficiency (25(OH)D levels <40nmol/L) in older people is high between 50- 75% especially in the northern latitudes and low levels have been found even in summer months[179;180]. A European epidemiological study showed the prevalence of vitamin D deficiency in older adults aged 71-76 years was 36% in older men and 47% in older women[181]. It is recommended that 25(OH) D levels <40nmol/L requires supplementation and 25(OH) D levels of >75nmol/L is the level for optimum bone and muscle health[182]. The recommended daily intake of vitamin D is between 400IU-600IU per day which may be inadequate to raise serum vitamin D levels to a desirable level >70nmol/L[183;184]. Studies have shown that in order to achieve optimal levels of 75-100nmol/L of 25(OH) D doses of between 700-1000IU would be needed. In the US, fortification of food such as milk and orange juice is mandatory, whereas in the UK only margarine is fortified with vitamin D. The

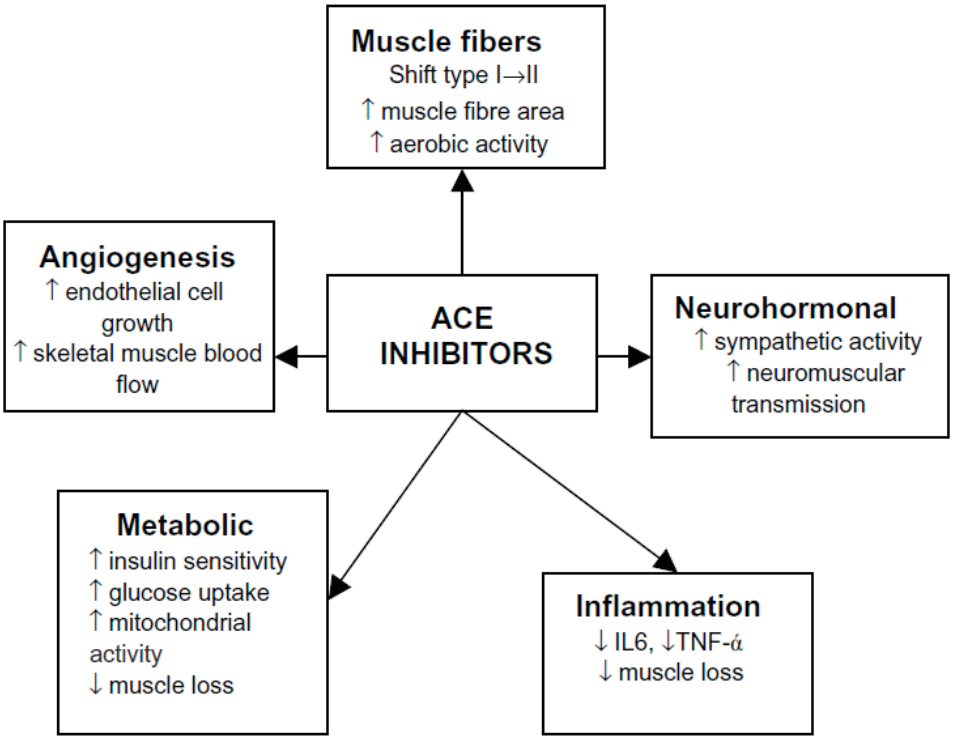
question of whether it should be mandatory for UK food products to be fortified with vitamin D remains controversial.

Although it is plausible to associate low levels of vitamin D with a reduction in muscle strength and physical function, the evidence for supplementation has been inconsistent. Safety issues surrounding vitamin D supplementation in older people include increased risk of nephrolithiasis and hypercalcaemia[185]. Further large randomized controlled trials are required with a longer follow up period in order to assess the safety profile of vitamin D supplementation in older people before it is recommended as a treatment for sarcopenia in clinical practice.

ACE inhibitors

ACE inhibitors have long been used as a treatment in primary and secondary prevention in cardiovascular disease as well as secondary stroke prevention. There is a growing body of evidence to suggest that ACE inhibitors may have a beneficial effect on skeletal muscle. ACE inhibitors may exert their beneficial effects on skeletal muscles through a number of different mechanisms (Figure 1.8). ACE inhibitors may improve muscle function through improvements in endothelial function, metabolic function, anti-inflammatory effects, and angiogenesis thereby improving skeletal muscle blood flow[186;187]. They can also increase mitochondrial numbers and IGF-I levels thereby helping to counter sarcopenia[188]. People with the type II genotype of the ACE gene who have low serum ACE levels show an increased response to physical endurance[189;190]. Therefore, lowering serum ACE levels with ACE inhibitors may have a beneficial effect on physical function.

Figure 1.8 – Effects of ACE inhibitors on skeletal muscle



Observational studies have shown that the long term use of ACE inhibitors was associated with a lower mean decline in muscle strength and walking speed in older hypertensive people[191;192]. Several studies have shown that ACE inhibitors improved exercise capacity in both younger and older people with chronic heart failure (CHF)[193-195]. Although this could be largely attributed to improvements in cardiac function, skeletal muscle atrophy is also associated with CHF so the evidence in muscle gains should not be discounted.

Few interventional studies using ACE inhibitors for physical function have been undertaken. One study, looking at functionally impaired older people without heart failure, has shown that ACE inhibitors increase six minute walking distance to a

degree comparable to that achieved after 6 months of exercise training[196]. Another found that ACE inhibitors increased exercise time in older hypertensive men[197]. However, a study comparing the effects of nifedipine with ACE inhibitors in older people found no difference between treatments in muscle strength, walking distance or functional performance[198]. It is possible that frailer subjects with slower walking speeds, who have a tendency to more cardiovascular problems, benefit more. This is reflected in the fact that adults with severe peripheral vascular disease significantly increase their walking time following treatment with ACE inhibitors[199]. This again highlights the significance of ACE inhibition on physical performance albeit in variety of patient groups. However, further trials are needed with longer follow up periods, to establish whether ACE inhibitors can improve physical function with chronic ACE inhibitor use.

Summary

Sarcopenia is a global health concern. It is usually accompanied by physical inactivity, decreased mobility, slow gait speed and poor physical endurance. With a growing older population there is now great interest in developing approaches to counteract the effects of sarcopenia and thereby reducing disability and dependency. A summary of the potential treatment options for sarcopenia is shown in Table 1.2.

The evidence suggests that regular physical activity can prevent physical functional decline in old age. In particular, aerobic or resistance exercise training has shown to improve muscle strength with as little as one exercise training session per week. Resistent exercise training has shown to increase muscle CSA as well as increasing the number of type II (fast twitch) muscle fibres, which leads to overall improvement in muscle power and physical functioning. However, participation in regular exercise requires motivation by the individual which may be difficult in some older sedentary people. Therefore, non-exercise interventions may offer a useful alternative which I will explore in this thesis.

Table 1.2 Summary of treatment options for sarcopenia.

Intervention	Effect	Comments
Exercise		
Aerobic exercise	↑ cardiovascular fitness,↑ endurance ↑ mitochondrial volume and activity	Pros: overall beneficial effects on physical
Resistance exercise	↑ muscle mass and strength ↑ muscle protein synthesis, ↑ muscle fibre size ↑ physical performance	Cons: motivation to exercise remains low
Nutritional supplementation	Varying evidence for ↑ muscle mass and ↑ muscle strength	Pros: ensures good protein intake Cons: problems with adherence
Hormone Therapy		
Testosterone	Varying evidence for ↑ muscle mass and ↑ muscle strength	Cons: masculinisation of females, ↑ risk of prostatic cancer
Oestrogen	Poor evidence for ↑ muscle mass but not function	Cons: ↑ risk of breast cancer
Growth Hormone	Some evidence for ↑ muscle mass Varying evidence for ↑ muscle strength	Cons: adverse effects; fluid retention, orthostatic hypotension
Vitamin D	Variable evidence for ↑ muscle strength ↓ falls in nursing home residents	Pros: ↓ fracture risk Pros: possible cardiovascular benefits
ACE inhibitors	Some evidence for ↑ exercise capacity	Pros: other cardiovascular benefits Cons: renal function needs monitoring
Creatine	Variable evidence for ↑ muscle strength and endurance when combined with exercise	Cons: reports of nephritis
Myostatin antagonists	No trials in older people	

1.4 Renin-Angiotensin-Aldosterone System

Background

The renin-angiotensin-aldosterone system (RAAS) plays an integral role in the homeostatic control of arterial blood pressure, tissue perfusion and extracellular volume. In 1898 a substance synthesised in the renal cortex of a rabbit was discovered to result in a sustained increase in arterial blood pressure. The substance was thought to be a hormone secreted by the kidney and was later known as 'renin'[200]. This finding was reinforced when it was found that hypertension could be prompted by renin release due to renal ischaemia induced by clamping the renal artery. It was later shown that 'renin' caused the proteolytic cleavage of a precursor initially named 'angiotensinogen' which in turn, produces angiotensin I (Ang I) and angiotensin II (Ang II). Ang I is cleaved by a co-existing enzyme angiotensin-converting enzyme (ACE) which is required to generate the active peptide Ang II[201]. It was later discovered that Ang II stimulates the release of the hormone aldosterone from the adrenal cortex which is required for the regulation of sodium and potassium balance.

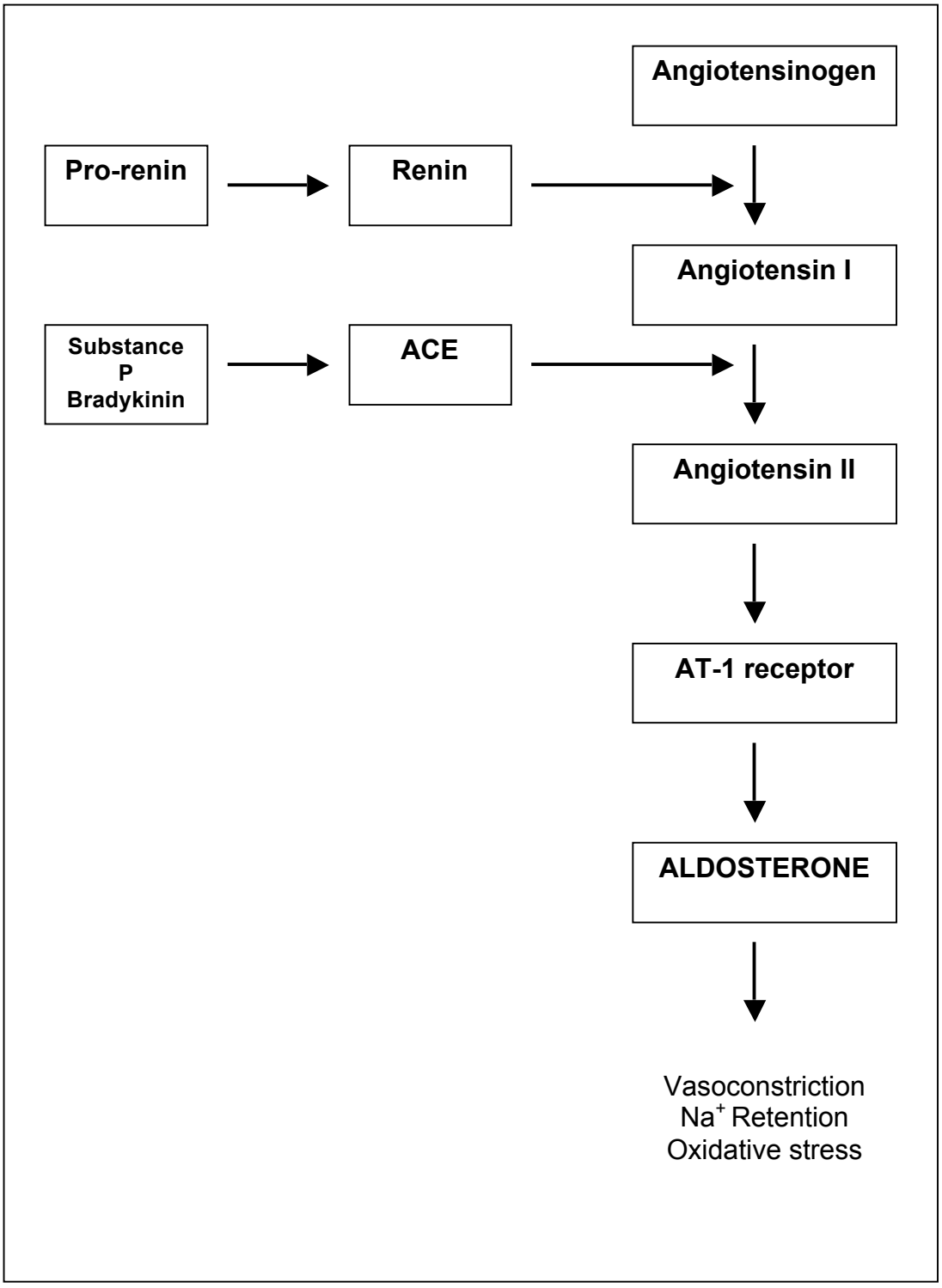
The classical RAAS pathway

The renin-angiotensin-aldosterone system (RAAS) is depicted in Figure 1.9. Evidence has shown that a reduction in circulating blood volume results in a reduction in renal perfusion pressure. Subsequently, this triggers the release of renin from the juxtaglomerular (JG) cells lining the afferent arterioles in the renal glomerulus. Renin is an aspartyl protease synthesised from the pre-hormone pro-renin. Renin is then stored in the granules of the JG cells, it is then stimulated by an exocytic process and released into the systemic circulation. Renin regulates the initial rate-limiting step of

the RAAS pathway by cleaving the N-terminal amino acids of circulating alpha 2 globulin angiotensinogen, to form the decapeptide Ang I. This in turn, is converted to the active octapeptide Ang II by removal of the C-peptide through the action of ACE. ACE is a membrane bound exopeptidase produced in the lung and vascular endothelial cell luminal membrane and locally in the glomerulus.

Ang II is one of the most important biological components of the RAAS and has a key role in maintaining circulatory homeostasis. Ang II primarily stimulates the release of aldosterone from the zona glomerulas in the adrenal cortex but it is also a potent vasoconstrictor. Ang II promotes vasoconstriction of the renal and systemic arterioles. ACE also helps regulate vasoactivity by inactivating bradykinin, a potent vasodilator. The potent actions of Ang II occur as a result of its binding to specific receptors (AT-1 and AT-2). Both act as G-protein coupled cell surface receptors but they differ in their effects. AT-1 receptors (divided into subtypes alpha and beta) are present in the cells of the target organs of Ang II and mediate most of the biological effects of Ang II including the secretion of aldosterone. Studies have shown the majority of the physiological actions of angiotensin II are mediated by AT-1 receptor. Two subtypes of AT-1 receptor have been identified AT-1 alpha (AT-1a) and AT-1 beta (AT-1b) receptors. The two receptors are thought to signal in a similar manner but are derived from two different genes and are expressed differently[202]. It appears that AT-1a receptor is found in most organs whereas AT-1b is more prominent in the adrenal and pituitary glands. In contrast AT-2 receptors appear to be present during fetal life in the brain, kidney and adrenal sites and levels appear to decrease in the postnatal period[203].

Figure 1.9 – The Renin-Angiotensin-Aldosterone System



Tissue RAAS pathway

There is now evidence to suggest that Ang II and aldosterone are synthesised in a variety of tissues in addition to the kidneys including the brain, heart, adipose tissue and blood vessels[202].

Much of local Ang II is synthesised when angiotensinogen penetrates from the plasma into the tissue. Angiotensinogen is enzymatically cleaved by renin which is either free or bound to the cell membrane. Alternatively, it has been postulated that other pathways for the synthesis of Ang II from angiotensinogen are present independent of renin. Studies have suggested that non-ACE pathways may be responsible for up to 40% of local Ang II[204]. Unfortunately, most of the evidence for the presence of alternative Ang II synthesis pathways derives from in vitro or indirect observational studies[205;206]. The presence of the alternative pathways in humans remains uncertain.

Aldosterone

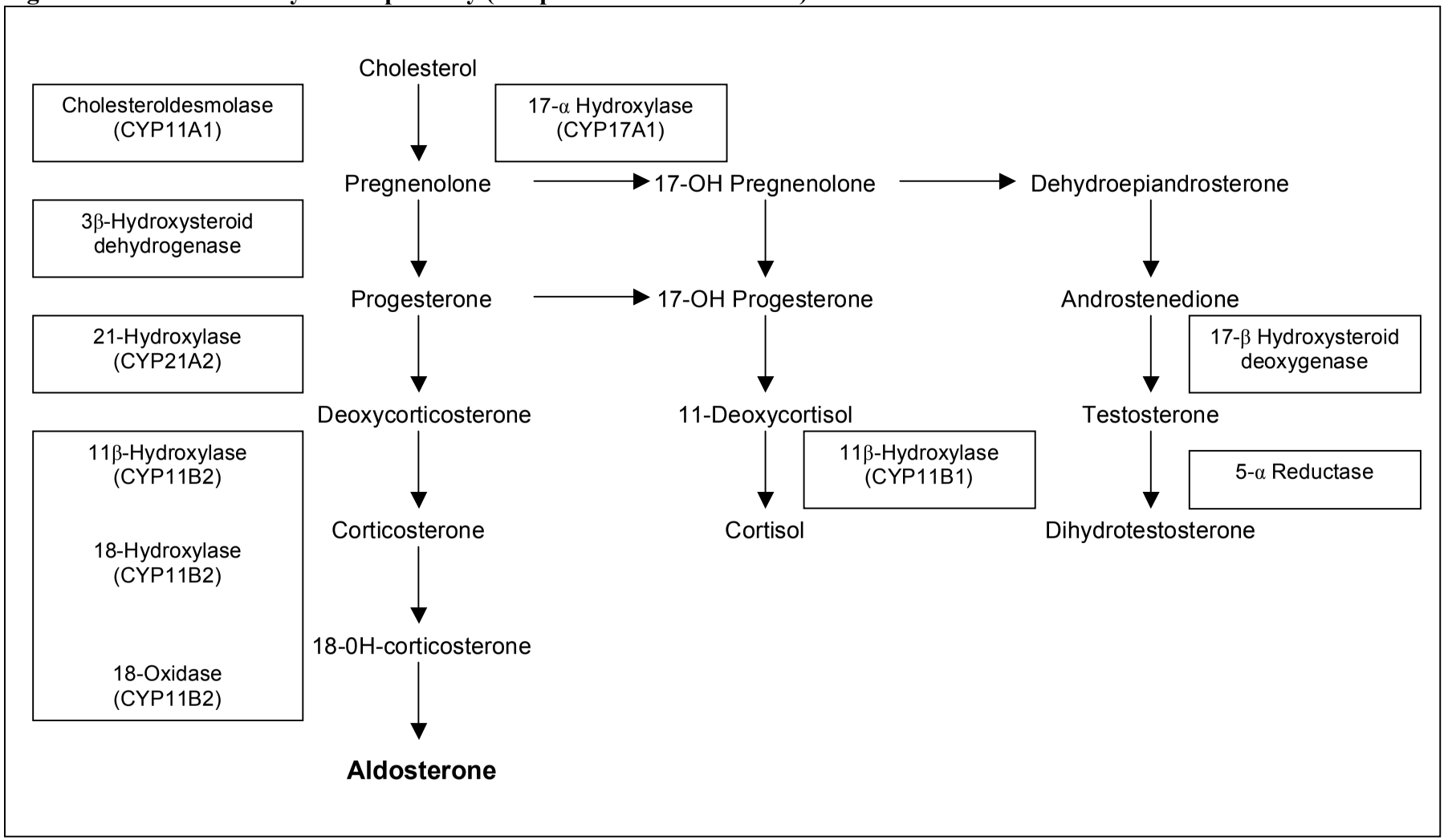
There is now increasing data to support suggestions that the adverse effects of Ang II are mediated through aldosterone. It is known that aldosterone is synthesised in the mitochondria of the zona glomerulosa cells of the adrenal gland. Cholesterol is converted to pregnenolone by a series of enzymes to progesterone, deoxycorticosteroid, corticosterone and lastly to aldosterone (Figure 1.10)[207]. Two forms of the P-450 cytochrome enzymes catalyse the final step in the synthetic pathway. These enzymes are encoded by two genes CYP11B1 and CYP11B2 which, although closely related, appear to differ in their activation and tissue distribution[208]. 11-beta-hydroxysteroid dehydrogenase (11β-HSD) catalyses 11-deoxycorticosterone (DOC) to corticosterone in the zona fasiculata and reticularis, under the regulation of

ACTH. Aldosterone synthase catalyses DOC to aldosterone in the zona glomerulosa[209]. Aldosterone synthesis is stimulated by Ang II, potassium and to a smaller extent adrenocorticotrophic hormone (ACTH). Aldosterone release is inhibited by somatostatin, atrial natriuretic factor and dopamine.

Classically, aldosterone binds to the mineralocorticoid receptor (MR) in the renal epithelial cells and stimulates the expression of genes encoding for aldosterone induced proteins. These proteins subsequently stimulate activation of sodium and potassium channels, mitochondrial adenosine triphosphate (ATP) and sodium-potassium ATP synthase (ATPase) (Na^+/K^+/ATPase). This leads to sodium retention potassium excretion and thus increases systolic blood pressure.

Aldosterone is an important regulator of blood pressure, with up to 15% of unselected patients with hypertension noted to have a high aldosterone/renin ratio[210]. It has been well known that a sustained increase in systolic blood pressure can cause numerous deleterious effects including tissue damage to the brain, kidneys and myocardium. Increasing levels of aldosterone and Ang II can also produce tissue damage directly by increasing blood pressure.

Figure 1.10. Aldosterone synthesis pathway (Adapted from White *et al*[207])



More recently studies have suggested that factors in early life can determine the control of aldosterone secretion. A cross-sectional study of 311 older men and women of 67-78 years of age, showed that patients with a low birth weight and high blood pressure had increased aldosterone levels, with patients with the highest aldosterone levels showing a 10mmHg difference in systolic blood pressure compared to those with the lower aldosterone levels[211].

There is growing evidence to suggest that aldosterone may be involved at extrarenal sites. Animal and human studies have identified the presence of the CYP11B2 gene which codes for aldosterone synthase enzyme, responsible for last step in aldosterone biosynthesis and 11β-HSD enzyme, involved in regulating the binding of aldosterone to the MR, at various sites including the brain, heart and vascular tissue[212-214]. Therefore, it is believed that aldosterone has paracrine actions. We know that high levels of aldosterone have deleterious effects on the cardiovascular system and other systems (Figure 1.11). In heart failure, high levels of aldosterone are closely associated with increased mortality[215]. Therefore, reducing aldosterone levels at a tissue level may have other beneficial effects.

Aldosterone induces myocardial fibrosis

Aldosterone promotes myocardial fibrosis and remodelling which are characteristic of left ventricular hypertrophy (LVH) and heart failure. Brilla *et al* showed that aldosterone induced myocardial fibrosis in the hypertrophied left ventricle and non-hypertrophied right ventricle in rat models, which was prevented by spironolactone without significant changes to blood pressure[216]. In human studies, patients with chronic heart failure (CHF) and hypertension, showed increased expression of

aldosterone synthase which was associated with an increase in myocardial fibrosis and LVH[217]. This suggests a role of locally produced aldosterone in the development of myocardial fibrosis. Plasma levels of procollagen type III amino peptide (PIIINP) are a useful marker of collagen turnover in the myocardium and hence a useful marker for myocardial fibrosis. The aldosterone antagonist, spironolactone has shown to reduce PIIINP levels in patients with CHF, suggesting that aldosterone promotes myocardial collagen formation[218].

Aldosterone induces vasculopathy

Aldosterone also promotes vascular fibrosis and induces tissues damage. In rabbit models of vascular damage, aldosterone administration enhanced neointimal thickening in the iliac artery and aorta. Spironolactone significantly inhibited the neointimal thickening, suggesting a role for aldosterone in neointimal proliferation[219].

It has been shown that aldosterone and Ang II produced tissue damage independently of their effects on blood pressure. Rocha *et al* suggested that aldosterone blockade by selective aldosterone blockers or adrenalectomy, significantly reduced the development of stroke and development of renal microvascular lesion, in salt-drinking, spontaneously hypertensive rats[220]. Importantly, these effects of aldosterone blockers occurred without changes in blood pressure. This suggests that aldosterone may play a role in vascular lesion development in the brain and kidneys and that theses effects are independent to its effects on blood pressure.

Aldosterone promotes autonomic imbalance

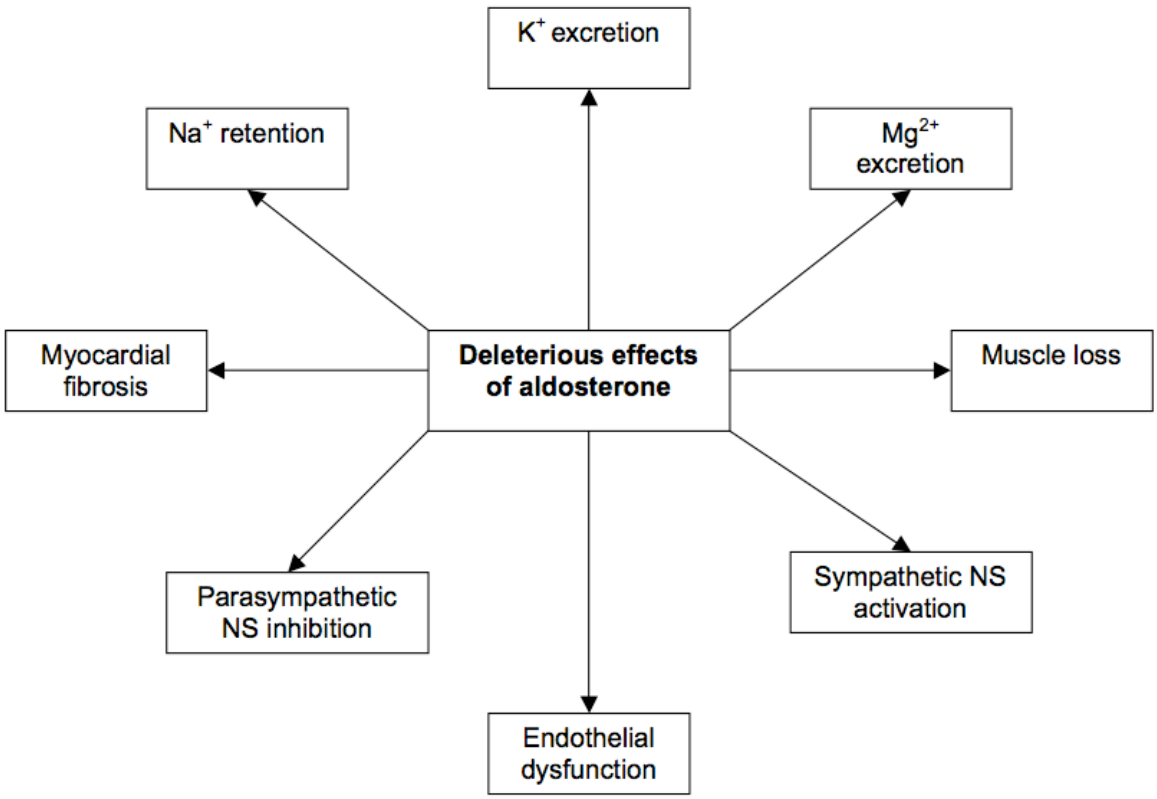
Autonomic balance involves the balance between the sympathetic and parasympathetic nervous system. Autonomic balance can be measured through baroreflex testing and heart rate variability. In a study in patients with CHF, the presence of autonomic dysfunction as measured by reduced heart rate variability was associated with increased mortality[221]. Aldosterone has shown to increase sympathetic activity and reduce parapsympathetic activity. In animal studies, Wang *et al* showed that aldosterone reduced parasympathetic activity by reducing the baroreceptor discharge from the carotid sinus and reduced heart rate response[222]. In patients with CHF, spironolactone reduced early morning heart rate and improved heart rate variability, suggesting spironolactone improves parasympathetic effects[218].

Aldosterone blockade

With growing evidence of wider extrarenal effects of aldosterone, finding methods to counter these patho-physiological effects has become clinically important. As aldosterone is a terminal hormone of the RAAS it would be feasible to suggest that pharmacological agents which interrupt this system proximally via ACE inhibitors or AT-1 receptor blockers would block aldosterone synthesis. However, recent *in-vivo* studies have shown this not to be the case. In a randomized evaluation of strategies for left ventricular dysfunction (RESOLVD) pilot study, patients with congestive heart failure were given both an ACE inhibitor and AT-1 receptor blocker[223]. Aldosterone levels fell within the first 17 weeks. However, the mean aldosterone levels subsequently returned to baseline by 43 weeks. We know that ACE inhibitors block the formation of Ang II by inhibiting the conversion of Ang I to Ang II. However, despite blocking Ang II, aldosterone levels can still rise in a phenomenon known as

'aldosterone escape'. Therefore, it may be impossible to completely block aldosterone inhibiting Ang II through ACE inhibition. This may be because ACE inhibitors eventually increase serum potassium levels and potassium is a powerful secretagogue for aldosterone release. Aldosterone is stimulated by both Ang II and potassium. However, ACE inhibitors only reduce Ang II levels and in fact increase potassium levels. The question now is whether another therapy directed at RAAS overactivity, in particular aldosterone receptor blockers, can have a greater effect at countering other extrarenal effects such as tissue damage.

Figure 1.11 – The deleterious effects of aldosterone



1.5 Evidence suggesting a role for aldosterone blockade in muscle function

It is possible that aldosterone blockade with spironolactone may well deliver benefits in conditions other than heart failure. In particular, aldosterone blockade with spironolactone may play a key role at maintaining muscle function and preventing physical decline in later life, which is the central focus of this thesis.

Aldosterone blockade reduces myocyte apoptosis

We know that prolonged activation of the renin-angiotensin-aldosterone system is the hallmark for many cardiovascular diseases including hypertension and heart failure, where it is believed that cell death may contribute to ventricular damage. Catecholamines, Ang II and aldosterone are all capable of causing cardiomyocyte death. The loss of cardiomyocytes leads to loss in cardiac structure and function resulting in a reduction cardiac reserve capacity[224].

Experimental studies in Wistar rats have shown that infusion with aldosterone increased the risk of both cardiac myocyte apoptosis as well as skeletal myocyte apoptosis. In the study, spironolactone was shown to reduce cardiac myocyte apoptosis by 90% and reduce skeletal myocyte death of soleus muscle in normotensive rats by 79%[225].

The mechanism by which aldosterone induces apoptosis is not yet fully understood. However, it has been suggested that the apoptotic effect of Ang II may be due to increased production of aldosterone which can up regulate AT-1 receptor

expression[226]. As skeletal muscle myocytes are known to express AT-1 receptors, it could be speculated that over-activation of the RAAS may contribute to myopathy. This demonstrates the potential role of spironolactone in reducing myocyte apoptosis.

We know that myocyte apoptosis occurs in the skeletal muscles of patients with chronic heart failure in a phenomenon known as 'cardiac cachexia'. This in turn can lead to muscle wasting, weakness and reduced exercise tolerance in a process similar to sarcopenia[227;228]. The plasma concentration of aldosterone in CHF patients with cachexia is three fold greater compared to age-matched non-cachectic patients without heart failure[229]. It may be postulated that the aldosterone receptor antagonist spironolactone may prevent the adverse effects of aldosterone on skeletal muscle, which includes, myocyte apoptosis, muscle wasting, muscle weakness impaired exercise tolerance, which are hallmark features in the process of sarcopenia.

Aldosterone blockade effects muscle metabolism

Aldosterone increases the re-absorption of sodium and chloride from the renal tubules. It also increases the excretion of potassium and independently causes loss of magnesium from the body by increasing urinary magnesium output. Aldosterone receptor blockade with spironolactone may improve muscle contractility by increasing skeletal muscle magnesium. It has been well documented that patients on long-term diuretic therapy have substantial losses of urinary potassium and magnesium[230]. Patients with CHF and arterial hypertension often have electrolyte disturbances. The addition of conventional diuretics, including loop and thiazide diuretics can further add to these electrolyte disturbances and possibly lead to serious adverse effects including cardiac arrhythmias.

Magnesium plays an important role at a cellular level and is used in many metabolic processes requiring the hydrolysis of phosphate groups. The energy required to activate the Na^+/K^+ pump on the cell membrane is provided by the hydrolysis of ATP, which requires magnesium to act as a co-factor. This process is essential for muscle contractile performance. Dyckner *et al* demonstrated that aldosterone blockade with spironolactone, increased both skeletal muscle magnesium and potassium levels, in CHF patients already on long-term potassium and magnesium wasting diuretics[230].

Patients with alcoholic liver cirrhosis have impaired functional capacity as a result in loss in muscle strength. This may occur due to a reduction in muscle magnesium and muscle potassium which deteriorates with increasing severity of liver disease. Alcohol ingestion appears to double renal excretion of magnesium along with secondary aldosteronism and poor nutritional intake can lead to further magnesium depletion.

A study by Aagaard and colleagues demonstrated that patients with alcoholic liver cirrhosis showed deterioration in muscle strength with a reduction of up to 41% in comparison to the control group[231]. The study also showed that there was a significant reduction in muscle magnesium and to a lesser extent muscle potassium levels. The addition of spironolactone treatment over a two week period increased muscle strength, muscle magnesium and contents of Na^+-K^+ pumps. Muscle potassium levels were not significantly different. This would indicate that improvements in muscle strength by spironolactone may be accounted for by its action on increasing muscle magnesium. The importance of muscle magnesium on muscle strength was also supported in an earlier study by Gullestad and colleagues who demonstrated that the

addition of oral magnesium supplementation to the diet of chronic alcoholics significantly increased hand grip strength[232].

It is possible that spironolactone could improve muscle strength and muscle contractility by increasing skeletal magnesium levels. It is this rise in magnesium levels which is thought to facilitate ATP production in skeletal muscle and thus increase contractility in skeletal muscle. Spironolactone may also aid muscle contractility by increasing levels of muscle Na^+-K^+ pumps.

Aldosterone blockade reduces oxidative stress and improves endothelial function

Nitric oxide (NO) is a free radical synthesised by NO synthetase. Nitric oxide (NO) is a multi-functional molecule synthesised from L-arginine by a family of enzymes called nitric oxide synthases (NOS) which are found in skeletal muscles but also in the brain and vascular endothelium. Recent studies have shown that skeletal muscle is the main source of NO[233]. Endogenous NO released from the skeletal muscle modulates skeletal muscle function including; regulation of muscle metabolism, muscle contractility and regulation of skeletal muscle blood flow[234]. NO is produced at high concentration during increased activity to prevent muscle fatigue, however persistent high levels of NO may lead to oxidative stress and tissue damage[235]. NO inhibits muscle contractility and reduces isometric force, mediated through cyclic guanosine monophosphate (cGMP) and targets regulatory proteins. It also inhibits mitochondrial respiration and glycolysis, thereby promoting glucose uptake[233].

There are three different isoforms of NOS. Endothelial cell NOS (eNOS), neuronal NOS (nNOS) which are present in skeletal muscle and inducible NOS (iNOS) which is normally expressed at low levels in skeletal muscle and increased during inflammation[236]. The eNOS and nNOS isoforms are involved in regulating skeletal muscle contraction, glucose uptake and metablolism[237]. The nNOS isoform is located in the sarcolemma of muscle fibres and is expressed at higher levels in fast-twitch muscle fibres when compared to slow-twitch muscle fibres. The eNOS is associated with mitochondria in mammalian muscle and is found in the endothelium of large blood vessels.

There is growing evidence that ageing alters the expression of NOS isoform profile with ageing increasing the iNOS/nNOS ratio shifting the function from contractile to inflammatory[238]. Studies have shown that an overproduction of NO and iNOS may be involved in muscle wasting, inflammatory myopathies and cachexia[239;240]. The mechanism is still poorly understood. It has also been suggested that nNOS expression in skeletal muscle is reduced during extreme disuse which might suggest that a similar process may occur in sedentary or physically disabled older people. Chronic inhibition of NOS can result in a 40% reduction in muscle CSA, a 30% loss of muscle mass as well as a reduction in walking speed[241].

The endothelium is responsible for maintaining a balance between vasoconstriction and vasodilatation of blood vessels including blood supply to skeletal muscles. If this balance is upset endothelial dysfunction occurs, causing damage to the arterial wall. Endothelial dysfunction occurs where there is a reduction in NO bioactivity and an increase in free radical production. Endothelial function declines with age, due to

blunting of vasodilator responses in vessels. Ageing has been associated with a reduction in expression of eNOS proteins in feed arteries of the soleus muscle in rats, thus reducing endothelial dependent vasodilatation[242].

Aldosterone may enhance vascular endothelial dysfunction by reducing nitric oxide bioactivity. This was demonstrated when healthy human volunteers were infused with aldosterone which did not alter blood pressure but did cause endothelial dysfunction. This phenomenon was called 'aldosterone induced vasculopathy'[243]. A similar effect might be seen in ageing, hypertension and atherosclerosis where there is inadequate NO bioavailability[244].

Aldosterone may reduce NO bioactivity by increasing nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, inducing free radical production which leads to the degradation of NO. NADPH oxidase is a major determinant of vascular tissue redox state which, in turn, is influenced by neuro-hormones such as angiotensin II, aldosterone and catecholamines[245]. NADPH oxidase catalyses the reduction of oxygen to superoxide anion which, reacts with NO to form reactive NO (peroxynitrite) resulting in oxidative stress.

The importance of aldosterone blockade on oxidative stress was demonstrated in a rabbit model of dietary hyperlipidaemia which is associated with high levels of circulating aldosterone levels. The model showed a significant reduction of NADPH oxidase dependent free radical production in the vascular wall as well as improved endothelial dependent vasodilatation, when given the selective mineralocorticoid antagonist, eplenerone over six weeks[246]. A subsequent experimental study in a rat

model of heart failure showed that the addition of spironolactone and ACE inhibition reduced NADPH oxidase subunit p22phox levels and improved endothelial function more than ACE inhibitors alone[247]. This suggests a potential role of the RAAS in the development of structural and functional vascular changes, aldosterone antagonists may therefore enhance endothelial function and increase skeletal muscle blood flow required during periods of increased physical activity.

In a double-blind placebo-controlled trial, Farqhuarson and Struthers demonstrated that aldosterone blockade with spironolactone, improved forearm blood flow by improving endothelial function and vascular NO bioactivity, in 10 patients with CHF[248]. The study also showed that spironolactone reduced Ang I but not Ang II mediated vasoconstriction despite chronic ACE inhibition. It was suggested that spironolactone reduced the vascular response to exogenous Ang I but not Ang II by reducing vascular ACE activity, raising the possibility that aldosterone may exert a positive feedback effect on vascular ACE. Therefore, aldosterone antagonism may exert extra ACE inhibition over and above standard ACE inhibitor therapy. Both blood pressure and degree of heart failure, as measured by the New York Heart Association (NYHA) functional class, remained unchanged during the study. This suggests that the effects of spironolactone on endothelial function were not related to its systemic effects. A similar improvement in the calf blood flow was also seen in hypertensive patients without heart failure who were treated with spironolactone[249].

Although some studies have shown partial improvement in endothelial function in patients with heart failure, it appears that aldosterone blockade has less of a benefit on endothelial function in patients with angina or diabetes mellitus. We know that spironolactone is effective in blood pressure control in poorly controlled hypertensive

type II diabetic patients. However, it doesn't appear to improve endothelial function in this group of patients[250]. It is speculated that the beneficial effects of blood pressure lowering by spironolactone is offset by its tendency to worsen glycaemic control, increase Ang II and cortisol levels.

It has been suggested that the age-related decline in endothelial function, associated with a decline in vasodilator response, could contribute to impaired muscle blood flow and reduced oxygen delivery during exercise. Aldosterone blockade, with spironolactone, improves NO bioavailability and endothelial function in patients with heart failure. As endothelial dysfunction is common in older people it could be speculated that spironolactone may have similar beneficial effects in improving endothelial function, skeletal muscle blood flow and thus reduce physical impairment in older people.

Aldosterone blockade reduces proinflammatory cytokines

Inflammation may interfere with endothelial function through the action of proinflammatory cytokines by stimulating the release of nitric oxide and endothelium derived hyperpolarizing factor.

Tumour necrosis factor alpha (TNF-α) plays a key role in the initiation and amplification of the inflammatory cascade in many pathological processes including muscle decline with old age[251]. TNF-α is produced by the macrophages and T-lymphocytes and activates other inflammatory cells through the production of chemokines, the induction of adhesion molecules in endothelial cells and lymphocytes. It also induces the production of other cytokines involved in the inflammation process

including interleukins (IL)-α, IL-1β, IL-6 and granulocyte-macrophage colony stimulating factor (GM-CSF). It has been suggested that TNF-α blocks the activation of eNOS by interfering with the phosphorylation and directly degrading eNOS messenger RNA (mRNA), thus inhibiting NO release which is essential for vasodilatation of blood vessels. TNF-α also stimulates muscle apoptosis and is associated with lower muscle mass and strength which accompanies sarcopenia. High levels of TNF-α are also associated with impaired exercise tolerance in CHF patients[252]

Studies in normal rats have shown that the addition of 11-deoxycortisone, an aldosterone precursor, stimulated central mineralocorticoid receptors and subsequently caused a rise in plasma and tissue TNF-α[253]. Blocking the mineralocorticoid receptors with spironolactone reduced the release of TNF- α. This may suggest that spironolactone may have anti-inflammatory potential which may be beneficial in pathological processes including muscle decline in old age.

In a study involving *ex-vivo* activated human blood leucocytes from patients with chronic arthritic diseases using gene expression analyses, the transcription of several pro-inflammatory cytokines including TNF- α, interferon-λ, GM-CSF and interleukin-6 were suppressed with spironolactone[254]. Spironolactone also reduced in-vitro cytokine production by human peripheral blood mononuclear cells in patients with congestive heart failure[255]. These studies have shown an anti-inflammatory effect of spironolactone at a transcriptional level. Although both animal and transcriptional studies have shown that aldosterone blockade have anti-inflammatory effects there is a lack of evidence that this occurs in man.

The levels of inflammatory cytokines including TNF-α rise with age and are associated with low muscle mass, low muscle strength and reduced exercise tolerance which are common features of sarcopenia. Therefore it is possible that aldosterone blockade may inhibit these effects.

1.6 Evidence suggesting aldosterone blockade improves exercise capacity

Maximal oxygen consumption during peak exercise (VO_2) is an objective way of measuring exercise tolerance and is an important component of physical function. Peak VO_2 declines with age[256]. As a result older people function close to their maximal effort when carrying out sub-maximal tasks. A reduction in exercise tolerance is associated with increased mortality, morbidity and increased future disability[256].

Modulation of the RAAS can improve exercise tolerance in patients with CHF. Studies have shown that ACE inhibitors, when used alone or in combination with Ang II receptor blockers, can improve exercise capacity in patients with heart failure[194;228] and also in patients without heart failure[196]. However it is possible that inhibiting other neurohormones in the RAAS, such as aldosterone, may have similar effects.

Elevated levels of aldosterone are associated with impaired exercise tolerance in patients with CHF[257]. Cicoira and colleagues showed that aldosterone blockade with spironolactone, improved left ventricular function in CHF patients already on standard heart failure therapy and improved exercise capacity by increasing peak VO_2 levels[258]. Cicoira also highlighted that improvements in exercise capacity were seen with spironolactone over and above the use of ACE inhibitors at baseline.

The syndrome of clinical heart failure, in the absence of left ventricular systolic dysfunction and in the absence of cardiac valvular lesions, is known sometimes as diastolic heart failure (DHF). DHF commonly occurs in older people with a prevalence

of around 5.5% in older people aged 70 years or above in a community dwelling population[259]. Previous studies have shown that older patients with diastolic heat failure have a reduced exercise tolerance[260]. There is some evidence to suggest that patients with DHF have significant activation of the RAAS and that aldosterone may play a key role in this pathophysiological process by promoting collagen deposition and promote myocardial fibrosis. In a prospective trial involving 11 women aged 60 years and above with diastolic heart failure, there was an improvement in exercise capacity with a 8.3% increase in peak VO_2, improved after taking 25mg of spironolactone for 4 months. The study also showed improvements in quality of life and diastolic filling paralleled with a reduction in myocardial fibrosis[261]. The study showed no change in six-minute walk distance. However, the study sample size was small and lacked a control group.

The mechanism underlying these effects of aldosterone blockade on exercise capacity is still unclear. It is possible that the effects of spironolactone on exercise capacity may be attributable to improved cardiac function, by a reduction in collagen deposition at the myocardium, thereby altering left ventricular remodelling and improving left ventricular systolic function and cardiac output. However, an effect at a peripheral level cannot be ruled out. An earlier study by Cicoira, showed that elevated levels of aldosterone were associated with impaired exercise capacity without any changes to resting haemodynamics or muscle mass[257]. Spironolactone has been shown to improve vascular endothelial function[248]. It may be speculated that improvements in vascular endothelial function with spironolactone may promote vasodilatation and muscle blood flow during periods of exercise, and possibly improve exercise tolerance, thus preventing decline in physical function with age.

1.7 Safety profile of spironolactone

Spironolactone is a mineralocorticoid receptor antagonist which was initially developed over 30 years ago. Although its plasma half life is relatively short (1.6 hours), after oral administration spironolactone is converted in the liver to its active metabolites canrenone and canrenoate which have half-lives of between 17-22 hours. Spironolactone lacks first pass metabolism and appears to have good oral bioavailability of around 90%. When it was initially developed, spironolactone was classified as a potassium-sparing diuretic due to its effects on epithelial ion transportation. However, spironolactone also has other biological effects.

Spironolactone is efficacious in the treatment of patients with heart failure. It is also used to treat resistant hypertension, primary hyperaldosteronism and cirrhosis with secondary aldosteronism. Prescription rates for spironolactone have risen in recent years following results from the RALES study, which showed the benefits of spironolactone on mortality rates in patients with congestive cardiac failure[262]. More recently guidelines from the British Hypertension Society, recommended spironolactone as a fourth line treatment for hypertension[263].

Side effects exist with spironolactone use. The pro-gestational and anti-androgen effects include gynaecomastia, breast pain, abnormal menstrual cycles and impotence. In the RALES study, the incidence of gynaecomastia or breast pain in men was greater in those receiving spironolactone compared to those on placebo (10% spironolactone versus 1% in placebo group; $p<0.01$)[262]. Other adverse effects include gastritis or gastrointestinal disorders which occurred in up to 2% of patients on spironolactone[264].

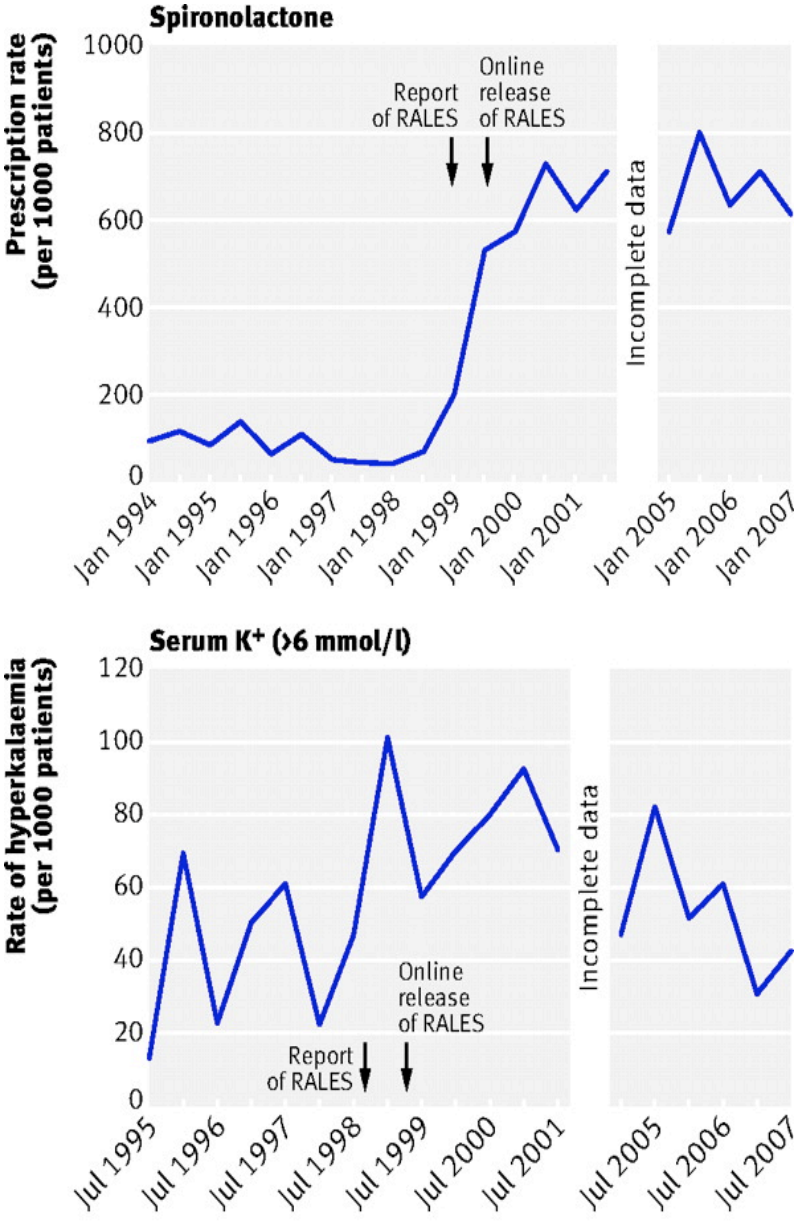
One of the most important side effects of spironolactone is an increase in serum potassium concentrations. In the RALES study severe hyperkalaemia, defined as serum potassium concentration >5.5mmol/L, increased with increasing doses of spironolactone from 5% in patients receiving 12.5mg/day to 20% in patients receiving 50mg of spironolactone per day[262]. In patients with reduced glomerular filtration rates, worsening heart failure or dehydration the risk of serious hyperkalaemia whilst taking spironolactone is greater. One study showed that more than 25% of patients were found to have serious hyperkalaemia (serum potassium >5.2mmol/L). Another study found that over 10% of patients treated with a combination of an ACE inhibitor and spironolactone were found to have serious hyperkalaemia. Patients who are more susceptible to hyperkalaemia appear to be older with a poorer baseline renal function. Therefore, long-term monitoring of serum potassium and renal function is required.

Following the outcome of the RALES study, there was an increased use throughout the developed world of spironolactone in heart failure patients concurrently treated with ACE inhibitors. In a Canadian observational study, the increased use of spironolactone in these patients was paralleled with an increase in hospital admissions and subsequent deaths from serious hyperkalaemia[265].

Following this a similar observational study was conducted in the Tayside area. The study evaluated prescription rates for spironolactone, rates of hyperkalaemia and renal impairment associated with spironolactone. Despite an increase in the prescription of spironolactone there was no increase in hyperkalaemia or renal toxicity. In contrast to the Canadian study hospital admissions for hyperkalaemia and outpatient hyperkalaemia actually fell (9.9 per 100 patients in 1999 to 2.9 per 100 patients in

2007) (Figure 1.12)[266]. This shows that spironolactone can be a relatively safe drug if patients' electrolytes and renal function are carefully monitored. Spironolactone is also well tolerated in a variety of patients including older people for which this thesis focuses on.

Figure 1.12 - Rates of spironolactone prescriptions and associated hyperkalaemia (Taken from Wei et al[266])



1.8 Summary

With a growing ageing population decline in physical function has become a major public health issue, as it is associated with disability in later life. Although regular exercise has been shown to increase muscle strength and slow functional decline, the majority of the older population are either unable or unwilling to contemplate adequate exercise participation. Alternative interventions are needed to improve physical function in later life.

There is now growing evidence to suggest that blocking the RAAS may help improve physical function in frail elderly people[196]. Previous randomised controlled trials have shown an improvement in physical function with ACE inhibitors in older people with and without heart failure[194;196]. Initially the adverse effects were initially thought to be mediated through activation of Ang II. However, it is now speculated that the effects of Ang II are mediated downstream via aldosterone.

Recent evidence suggests a possible role for the aldosterone receptor blocker, spironolactone, in improving physical function in older people. Firstly spironolactone may improve muscle function by: reducing skeletal myocyte loss[225] and improving skeletal muscle contractility[231]. Secondly spironolactone may improve vascular endothelial function, enhance skeletal muscle blood flow and inhibit pro-inflammatory cytokine production[255]. Finally in a randomised controlled trial, the use of spironolactone resulted in improved exercise capacity over and above that achieved with standard ACE inhibitor therapy in patients with heart failure due to LV systolic impairment[258]. Although this improvement may have occurred due to improved cardiac function, it is possible that spironolactone, an inexpensive, widely used medication,

may also have direct effects on skeletal muscle. A study was therefore designed to evaluate the effect of spironolactone on physical function in functionally impaired older people without heart failure.

2. METHODS

2.1 Study Design

This study was a randomised, double blind parallel group placebo controlled trial. The study was approved by Tayside Committee on Medical Research Ethics (reference number 08/S1402/34) and was funded by the Chief Scientist Office (CBZ/4/635) as shown in Appendix A. This study conformed to the Declaration of Helsinki. The aim of the study was to examine whether inhibition of the renin-angiotensin- aldosterone system (RAAS) with spironolactone would improve physical function in older people without heart failure.

Figure 2.1 shows the inclusion and exclusion criteria used in the study. Participants aged 65 years and above were eligible if they had self-reported problems of activities of daily living. Self-reported functional dependence was assessed by asking participants questions using the Barthel Index which was documented on a screening proforma (see Appendix A). The Barthel Index consists of 10 items describing activities of daily living and mobility. The 10-items include the need of assistance in each of the following tasks; dressing, grooming, feeding, transferring from chair to bed, bathing, walking, toileting, climbing stairs as well as the presence or absence of faecal or urinary incontinence. Participants were eligible if they were dependent in one or more of the 10 items of the Barthel Index. The Barthel Index was initially introduced by Mahoney and Barthel in 1965 and is now a widely used measure to assess functional status in rehabilitation[267]. It was initially developed for the assessment of patients with neuromuscular and musculoskeletal disorders. It has subsequently been used to measure functional change in rehabilitation of patients who have had a stroke. Now it is widely used for assessing

functional status in older people. It has good intrarater reliability (0.95) and good test-retest reliability (0.89) and has been used to assess treatment outcomes in numerous studies[268].

Participants with cognitive impairment (MMSE<20/30) were excluded as they are less likely to give informed consent. Participants were also excluded if there were wheelchair bound or requiring assistance to mobilise, as outcome measures such as six minute walk test are designed for ambulant people.

Figure 2.1 – Recruitment criteria

Recruitment criteria

Inclusion criteria

- Aged >65 years old
- Self reported problems with activities of daily living

Exclusion criteria

- A clinical diagnosis of symptomatic heart failure (according to European Society of Cardiology guidelines)
- Asymptomatic LV systolic or diastolic dysfunction
- Already taking spironolactone, ACE inhibitors or Angiotensin II receptor blockers
- Previous reported intolerance to spironolactone
- Hypotension (systolic blood pressure <100mmHg)
- Cognitive impairment precluding informed consent (MMSE<20/30)
- Serum creatinine >200umol/L (eGFR<30ml)
- Serum sodium <130mmol/L
- Serum potassium >5.0mmol/L
- Symptomatic orthostatic hypotension
- Wheelchair bound

2.2 Recruitment

Participants were recruited through two different methods:

The Scottish Primary Care Research Network (SPCRN)

Participants were recruited using the East of Scotland node of the Scottish Primary Care Research Network (SPCRN) formerly known as Scottish Practices and Professionals Involved in Research (SPPIR). The network was established in 2002 to help co-ordinate national research activity in primary care. It is funded by the Chief Scientists Office, centrally managed by the Scottish School of Primary Care (SSPC) and operationally managed at a regional level in Scotland. It provides an essential link between researchers and primary care.

The East of Scotland node of SPCRN comprises of 30 GP practices who are interested in being involved in research, across Tayside and Fife across 8 LHCC areas. The co-ordinator from the East node of SPCRN contacted GP practices within the network by post to invite them to be involved in the study. Practices willing to be involved in the research project were visited by a SPCRN research officer. Using study eligibility criteria and the patient computer data base system within the practice, the research officer generated a list of potentially eligible participants. The list was then scrutinized by the practice GP to exclude patients who had suffered from a recent illness, recent hospitalisation or if they had died shortly after the patient list had been generated.

A letter of invitation along with a Patient Information Leaflet (PIL) (Appendices B and C), reply slip and a stamped addressed envelope were sent from the GP to patients who

were considered potentially eligible to participate in the study. Only those individuals who were interested to know more about the study were asked to reply. Individuals who replied were contacted by telephone by LB.

During the telephone screening, a brief but clear outline was given of the purpose of the study and details of participant's involvement within the study. Eligibility was also checked by asking individuals date of birth and asking whether they had problems with activities of daily living (ADLs). Individuals were encouraged to ask questions at any point during the screening.

Medicine for the Elderly services

Participants were also recruited from the Medicine for the Elderly outpatient services in Tayside. The medical records of patients attending the Medicine for the Elderly services, located at the Royal Victoria Hospital Dundee or Perth Royal Infirmary, were screened for potential eligibility to the study. Patients were either approached at the Day Hospital at either the Royal Victoria Hospital or Perth Royal Infirmary in person by LB or were posted a study information pack containing a letter of invitation to take part in the study, a PIL, reply slip along with a stamped addressed envelope.

All potential participants approached at the Day Hospital were given adequate time to study the PIL. Potential participants were contacted by telephone by LB within 7 working days to determine whether they were willing to participate in the study.

Patients attending the Day Hospital for physical rehabilitation were not approached until at least 4 weeks had elapsed after they had been discharged from the Day Hospital to avoid the risk of physical co-intervention which may introduce confounding variables.

Informed consent

Verbal consent

All potential participants gave verbal consent for LB to review their hospital medical records to further scrutinize eligibility for the study prior to arranging an appointment for baseline screening visit.

Written consent

All potential participants were given the opportunity to ask questions. All participants gave written informed consent at baseline screening visit. (See Appendix D for patient consent form).

2.3 Clinical assessments

Clinical assessment was performed at the point of baseline screening for eligibility to the study. The assessment comprised of:

- Past medical history
- Concomitant medication
- Use of walking aids (none, 1 or 2 sticks, zimmer frame or triwheel walker)
- Height (cms)
- Weight (Kgs)
- Physical examination
- Lying / standing blood pressure (mmHg)
- Echocardiograph

Lying and standing blood pressure

Blood pressure was measured using a standard automatic sphygmomanometer (A&D UA-779), which has been validated according to protocol of the European Society of Hypertension on the measurement of ambulatory blood pressure[269]. Participants rested in a supine position for 5 minutes before blood pressure was recorded. Measurements were recorded in the supine position and then standing after 30 seconds, 2 minutes and 3 minutes to measure for orthostatic hypotension[270]. Symptoms of dizziness and light headedness were noted.

Echocardiography

Echocardiography was performed by LB using the SonoSite® TITAN™ ultrasound system at baseline and 20 weeks in order to assess left ventricular (LV) systolic function at baseline and whether the participant had developed left ventricular dysfunction during the course of the study.

Participants were asked to position themselves in the left lateral position and the couch was tilted so that the participant was lying at 30^0. Images were viewed in the parasternal long axis, short axis, 4-chamber and 2-chamber view. A subjective assessment was made of left ventricular function to rule out LV systolic dysfunction. If LV systolic impairment was detected it was recorded as 'mild', 'moderate' or severe' impairment. Doppler studies were also used to estimate the pressure gradient across the aortic and mitral valves.

Subjective visual assessment of LV function has good reproducibility compared to other methods of echocardiography such as contrast ventriculography[271]. Visual estimation of LV systolic ejection fraction has correlated closely and reliably to more formal and time consuming methods such as Simpson's rule and wall motion score index[272]. The accuracy of subjective assessment of LV function has also been demonstrated using a hand held device in a busy outpatient clinic and was comparable to the accuracy of echocardiography using Simpson's bi-plane method, in patients with at least moderate, left ventricular dysfunction[273]. Visual assessment of LV systolic function has also been used in frail older patients with multiple co-morbidities[196;274].

2.4 Study visits

Baseline assessments

Baseline screening visits were carried out at either the Royal Victoria Day Hospital Dundee or Perth Royal Infirmary, depending on where the participants lived. After screening for eligibility and clinical assessments, baseline measures were taken for the primary outcome, the six minute walk test (6MWT) and secondary outcomes were change in; Timed-Get-Up and Go test (TGUG), Incremental Shuttle Walk test (ISWT), EuroQuol and Visual Analogue Scale questionnaire (EQ-5D and EQ-VAS), Hospital Anxiety and Depression Scale (HADS) and Functional Limitation Profile (FLP). Blood tests were also taken to measure serum urea and electrolytes (U+E's), magnesium, B-type natriuretic peptide (BNP), aldosterone and random cortisol.

Follow up assessments

Follow up assessments during the study are shown in Figure 2.2.

Home visits (weeks 2 & 5)

A follow up clinical assessment was carried out at the participant's home at 2 and 5 weeks after randomisation. During these visits safety bloods tests were taken for serum urea and electrolytes and serum magnesium to monitor for changes in renal function. Sitting blood pressure was also recorded to rule out hypotension. If the 12.5mg spironolactone or matching placebo dose was tolerated and renal function remained stable after 2 weeks, the dose was up-titrated to 25mg of spironolactone or placebo.

Participants with a rise in creatinine ≥20% of baseline values or serum potassium ≥5.2mmol, remained on the starting dose (spironolactone 12.5mg or placebo). Participants had their study medication withdrawn if serology showed a rise in creatinine >25% from baseline, serum potassium >5.5mmol, creatinine above 200μmol/L or their systolic blood pressure <100mmHg. However, participants remained in the study and underwent their remaining follow-up visits in order to preserve intention-to-treat analysis.

Return visits to Day Hospital (weeks 10 & 20)

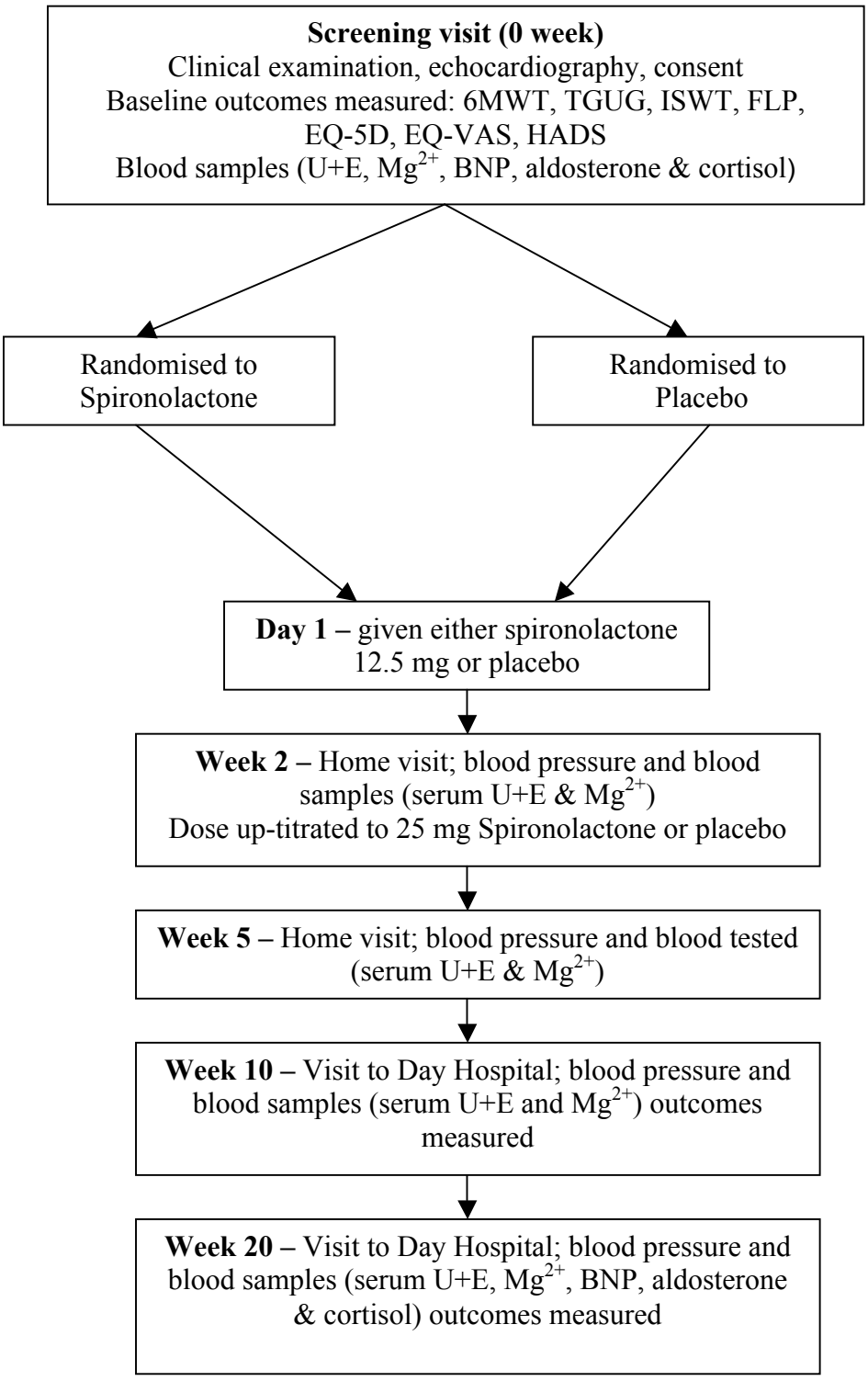
Participants returned to Day Hospital at weeks 10 and 20 for assessment of outcomes (6MWT, TGUG, ISWT, EQ-5D and EQ-VAS, FLP, HADS). Blood samples were also taken for BNP, aldosterone, cortisol, urea & electrolytes and serum magnesium. All assessments were done without reference to baseline values. At 20 weeks echocardiography was repeated.

Adverse events

All adverse events were reported in accordance with the Medicines for Human Use (Clinical Trials) Regulations 2004. At each study visit (baseline, 2 weeks, 5 weeks, 10 weeks and 20 weeks), patients were asked about any adverse events since their last study visit, including any hospitalisation, unscheduled GP visits or new disabilities. The sponsor was notified if there was a serious adverse event (SAE) or serious adverse reaction (SAR) except, if it was a known adverse drug reaction that was set out in the Summary of Product Characteristics of spironolactone.

A record was also kept of any changes to a participant's medication during the study.

Figure 2.2 – Flow diagram of study assessments



(U+E = Urea and electrolytes, Mg^{2+} = serum Magnesium)

2.5 Randomisation & medication dosage

Randomisation

Participants were given a sequential randomisation number on entry to the study and were given the medication with the corresponding randomisation number.

Randomisation of medicine was performed by Tayside Pharmaceuticals, Ninewells Hospital, who over-encapsulated the spironolactone and placebo in order to give them an identical appearance. The medication bottles were labelled with sequential patient numbers. Tayside Pharmaceuticals used a computer generated randomisation number table for randomisation. Block randomisation was done in batches of 20 in order to ensure equal group numbers between spironolactone and placebo. As randomisation was carried out by staff at Tayside Pharmaceuticals 'allocation concealment' was ensured.

Medication dosage

Participants were randomised to receive either spironolactone or placebo for the 20 weeks. The starting dose for medication was 12.5mg of spironolactone or placebo which was given on day 1 after screening. If tolerated this dose was then increased after two weeks to 25mg of spironolactone or placebo.

The medication was delivered to the participant at home by LB along with instructions on how the medication was to be taken. A contact telephone number was given to the participant and was also printed on a label on the medication bottle. The participant was advised to contact LB or their own General Practitioner if there were any adverse

effects. A letter was also sent to the participants GP informing them that the participant had commenced the study. A copy of the letter was also filed in the participants' medical records.

Medication adherence

Participants' ability to open childproof tops was assessed. Any participant who had difficulty in opening a child proof top was given a standard non-childproof bottle top.

Medication compliance was assessed by tablet counting at weeks 2, 10 and 20. The number of tablets returned was then documented in the participant's case report form (CRF) and in a drug accountability log, held by the Clinical Trials Pharmacist at Ninewells Hospital, Dundee.

2.6 Outcome measures

Primary outcome measure – Six Minute Walk Test

The primary outcome was the change in the distance walked in the six minute walk test (6MWT) from baseline over the twenty week period.

The 6MWT was conducted in a quiet enclosed corridor. The 25 metre course was marked at one metre intervals and chairs were placed at each end of the course. The participants were instructed to walk from one end of the course to the other for six minutes using their usual walking aid if needed. The participants were encouraged to exercise to the point of maximum ability but also were advised to rest if necessary. Participants only terminated the walk prior to 6 minutes if they experienced severe shortness of breath, dizziness, angina, or muscular pain. Standardized encouragement was given to every participant every 30 seconds. At the end of the six minutes the participants were asked to stop. The total 6MWT distance was recorded to the nearest metre. The 6MWT was performed at baseline and then repeated at weeks 10 and 20.

Functional walk tests are exercise tests that measure physical performance or exercise capacity as well as measuring the ability to undertake physically demanding activities of daily living[275]. They are considered to be an objective way to measure response to treatment and are reproducible in an older population compared to treadmill testing and cycle ergometry.

In 1968 a 12 minute run test was carried out in 115 healthy male US Air force Officers. The test correlated closely with maximal oxygen consumption (VO_2) and was therefore

a useful measure for physical fitness[276]. This was then modified to a 12 minute indoor walk test to disability in patients with chronic bronchitis[277]. Later the concept of a shorter 6MWT was developed in a similar population. It was found that decreasing the time to 6 minutes did not significantly reduce the reproducibility of the test. The 6MWT was also found to be better tolerated and less exhausting in patients with moderate respiratory disease than the 12 minute walk test[278].

The 6MWT has been widely used in a number of studies to measure functional status in patients with COPD, heart failure[279], peripheral vascular disease[280], fibromyalgia[281] and in older patients[282]. It has also been used for measuring response to medical interventions as well as a predictor for morbidity[283] and mortality[284].

The 6MWT has shown to be a validated measure of functional status in older people. Compared to other functional walk tests i.e. the self-paced walk test or the shuttle walk test, it appears to be better tolerated and more reflective of activities of daily living[285]. In frail older patients with heart failure a strong correlation was found between the distance walked in 6MWT and quality of life assessment as measured by the Chronic Heart Failure Questionnaire (CHQ). The study showed the 6MWT had good test-retest reliability with a high intra-class correlation (ICC) of 0.91[286].

The 6MWT has also shown to be a useful integrated measure of physical activity in a community dwelling older population, with a significantly greater increase in distance walked for physically active older adults compared to those who are less active ($p<0.001$). It has moderate correlation to other measures of physical function such as chair stands ($r=0.67$) and gait speed ($r=0.73$)[287]. Recent studies have shown the 6MWT

to be a useful measure of frailty in older adults with heart failure, frailer patients with lower endurance walked a shorter 6MWT distance. The study not only highlighted the use of the 6MWT in identifying frailer patients but it also showed the 6MWT to be a useful measure at identifying individuals with intermediate frailty who maybe in transition to becoming frail[288]. This demonstrates the utility of the test in predicting disability and, more importantly, at identifying patient's risk of future frailty who may benefit from interventions aimed to prevent decline to frailty.

Although the 6MWT has been widely used to measure physical performance in older people few studies have established the 'normal ranges' for 6MWT distance in normal healthy elders. In one study 700 metres was suggested to be a 'normal' 6MWT distance in healthy adults but did not specify whether this was applicable for all ages[289]. A subsequent study in healthy older subjects showed that adults aged 50-85 years had a mean 6MWT distance of 631$\pm$93 metres. However, the study showed considerable variability in the 6MWT distance ranging from 383-820 metres[290]. Multiple regression analysis showed that age, height, sex and weight were independent contributors of the 6MWT distance and gave 66% of the variability. Therefore, it is difficult to fix the normal range of the 6MWT in older adults without taking these factors into consideration. In 2002 the American Thoracic Society published guidelines for the 6MWT and stated that the distance covered in the 6MWT was influenced by a number of factors (Figure 2.3)[291].

Figure 2.3 - Factors reducing the 6MWT distance (taken from 2002 ATS guidelines[291])

Factors reducing the 6MWT distance

Short stature

Older age

Increased body weight

Female sex

Cognitive impairment

A shorter corridor (more turns)

Chronic diseases – pulmonary disease (COPD, asthma, cystic fibrosis)
- cardiovascular disease (angina, MI, CHF, PVD)
- musculoskeletal disorders (arthritis, muscle wasting)

Factors increasing 6MWT distance

Taller height

Male sex

High motivation

Medication for disabling disease taken immediately prior to test

Oxygen supplementation for exercise induced hypoxaemia

Patients who previously performed the test

In a large prospective Cardiovascular Health Study healthy older adults aged >68 years were asked to perform the 6MWT along with other performance based measurements (gait speed, chair stands and grip strength). The study showed a shorter 6MWT distance corresponded to older age, higher weight, larger waist circumference and weaker hand grip strength[282]. Factors of psychological health such as depression were also found to correlate to 6MWT distance[292].

A negative correlation was also found between 6MWT distance and age (r=-0.42) with subjects aged ≥75 years covering a shorter distance than those ≤65 years ($p<0.05$) regardless of their health status[293]. Other studies have shown that in healthy older people there was a moderate degree of negative correlation between a cognitive function and 6MWT distance. Encouragement can also influence the 6MWT distance. Guyatt *et al* showed that standardised encouragement at regular intervals compared to no encouragement produced a change in 6MWT distance of 20 metres which was statistically significant[294]. The layout of the test also plays an important role in the 6MWT distance. Individuals who performed the test in a considerably shorter corridor had a much lower mean 6MWT distance[295]. This is probably due to the fact that participants lost additional time on turning more frequently on a shorter course. This demonstrates the need for the same protocol on repeated assessments.

As well as being a useful measure of function and predicting disability the 6MWT has also shown to provide valuable information regarding prognosis. Studies involving patients with left ventricular dysfunction have shown that individuals who walked <300 metres in the 6MWT had a 3.7-fold increased risk of dying than those who walked

longer distances[284]. A 6MWT distance of <300 metres also predicted an increase in severity of heart failure as well as a reduction in ability to perform daily activities[296].

The 6MWT has good correlation with other objective measures of aerobic exercise capacity such as oxygen uptake at peak exercise ($V0_2$) previously determined through treadmill of cycle ergometry tests. Compared to these established measures of aerobic exercise capacity the 6MWT was much easier to perform and more closely related to daily physical activities in older people[275;279]. Many old, frail and severely limited patients were physically unable to perform standard maximal cycle ergometry or treadmill exercise testing. Individuals with walking aids were simply unable to participate. Therefore, this excludes a large proportion of people with functional impairment. Consequently, the 6MWT is a more inclusive method of measuring aerobic exercise capacity for older people. Although the endurance shuttle walk test appears to be a promising measure of maximal aerobic exercise capacity in patients with chronic respiratory problems[297], it lacks validity in older people.

Hand grip strength has shown to be a direct measure of skeletal muscle and disability[298]. In the Cardiovascular Health Study cohort grip strength remained a strong independent predictor of the 6MWT distance in both older men and women. The study showed the mean grip strength for women was 23Kg and 40Kg for men in the 65-69 year age groups. A 10Kg increment in grip strength in both men and women equated to an increase of 14m in the distance walked in 6MWT[282]. This demonstrates the use of the 6MWT as measure of muscle strength.

The repetition of a test within a short period of time can produce a learning effect. The 6MWT tends to increase with test repetition for example, a learning effect occurs due to test familiarisation. The magnitude of this learning effect appears to vary widely between studies from 4.5% to 33% of the initial distance walked[32]. Results vary between the first and second repeated walk test but appeared to plateau from the third repeat onwards, in the absence of any change in clinical state. In a study involving 50 healthy, young volunteers the initial learning effect was maintained during a 2-month period and that only a modest learning effect occurred with repeated testing (practice walks) on follow-up visits[299]. It is not known if results taken from studies involving young healthy volunteers could be extrapolated for older people recruited to clinical trials. For the purposes of this thesis it was decided that the use of practice testing would be difficult to perform in older people with mobility problems and functional impairment.

The 6MWT has been shown to be responsive and so is a good measure of change in functional status over time. It has also been used to measure the treatment effect for both pharmacological and non-pharmacological interventions. A randomized controlled trial which recruited older people in retirement villages, involved intervention of a 12-month exercise program, combining strengthening and aerobic exercises. The intervention group maintained their level of physical function and showed a reduction in falls by 22%. However, the control group showed a mean reduction in the 6MWT distance of 15 metres[300]. In a 6 month single-blinded randomized controlled trial older people with self-reported functional impairment were assigned to a home based progressive strength, balance and general physical activity group with encouragement.

The intervention group showed an 18 metres improvement in 6MWT compared to the control group.[301]

In a double-blind randomized controlled trial the 6MWT was used to assess the response to treatment with perindopril in older patients with left ventricular systolic dysfunction. The treatment group of the study showed a 37.1m significant improvement in 6MWT distance compared to placebo (-0.3m) ($p<0.01$) after a 10-week period[194]. A similar response to perindopril was also seen in a group of older functionally impaired non-heart failure patients. Results from the study showed a mean difference in 6MWT of 31.4m (95% CI 10.8m to 51.9m, $p<0.003$) between the treatment and placebo group[196]. For the purposes of this thesis a change in 6MWT distance of 30 metres was considered a clinically relevant change in an older population with functional impairment.

The current literature supports the use of the 6MWT as a sub-maximal exercise test to measure physical performance in a variety of people including those who suffer with chronic heart failure, COPD and in older adults. The 6MWT may also be suitable in elderly patients who find it difficult to complete adequate treadmill exercise tests[279;286]. It is a simple, inexpensive performance based measure. It is particularly useful in assessing functional status in older people as it reflects the capacity to perform daily activities. More importantly, it is a reproducible test which is responsive to change and has been useful to assess response to intervention in an older population[302].

Secondary outcomes

The "Timed-Get-Up and Go" test (TGUG)

The "Timed-Get-Up and Go" test (TGUG) is a useful tool for measuring clinically significant changes in the mobility of frail patients. It is a useful measure of explosive muscle power rather than endurance. The time taken to complete the task reflects the degree of functional impairment.

The participant was instructed to sit with their back against the upright of a standard armchair (measuring 46 cms from the seat to the ground), with their arms rested on the arms of the chair. At the word 'Go' the participant got up from the chair, using their hands to push up from arms of the chair, walked a 3 metre distance at their normal pace, turned around and returned to their seated position. Participants were encouraged to use their own walking aids if required. The total time taken to perform the task was recorded in seconds.

The original 'Get-Up and Go' test was developed by Mathias and colleagues in 1986 to measure balance. The performance was graded on a 5-point scale but was found to be too subjective. In order to overcome this subjectivity, the test was modified to include the time taken to perform the task (TGUG)[303]. It is a simple short test to assess basic mobility especially in patients with functional impairment[304].

The test incorporates everyday movements and is a useful method in assessing global functional status. Firstly, rising from a sitting to standing position requires both strength and technique. Secondly, walking a path of 3 metres requires both acceleration and

deceleration as a participant prepares to turn. Finally, turning around to sit requires balance and orientation while adapting body position to the chair.

It also correlates with other established assessment tools for activities of daily living such as the Barthel Index (r=-0.78)[304]. In clinical practice, the TGUG test has been widely used in the functional assessment of older people and has shown to be a reliable method for predicting falls risk [305;306]. It is currently recommended by both the American and British Geriatrics Societies as a screening tool for falls risk[307].
The TGUG test has shown to have good test-retest reliability in several specific populations including community dwelling older adults[304;306], people with Parkinson disease[308], older people with unilateral limb amputation[309] and those with chronic stroke disease[310]. However the test has been reported to have poorer test-retest reliability in patients with cognitive impairment[311]. In a cohort of cognitively impaired (mean MMSE 18.7$\pm$5.6) participants in a residential home, the addition of verbal cuing during TGUG performance, helped patients overcome any variability of the test[312]. This demonstrates the potential versatility of the test in older people with cognitive impairment.

As well as being a useful tool for predicting risk of falls, balance problems and risk of future institutionalisation[313]. The TGUG test is a simple, inexpensive assessment tool for physical function[314]. The TGUG test has shown good validity when compared to other measures of functional status such as gait speed, Berg balance test and Barthel index[304].

The more time taken to perform the test indicates a greater degree of functional impairment[304]. A wide range of TGUG scores have been reported in studies involving

older people. In a study involving both community-dwelling and institutionalised older patients, individuals who performed the TGUG test >12 seconds were more likely to suffer from mobility impairment[315]. However, 9% of the institutionalised patients were unable to perform the test[306]. The TGUG is a sensitive and specific measure of predicting older adults who are at risk of falling. In one study 30 community dwelling frail elders who took >14 seconds to perform the TGUG were found to be at greater risk of falling but this result was not verified in other studies mainly due to the fact that individuals in the study did not have any underlying pathology[316]. In contrast, older adults with underlying neurological pathology required >30 seconds to complete the task. They were more dependent in activities of daily living and scored lower in the Berg Balance Scale[304]. In a large meta-analysis of 21 studies reporting TGUG scores in older healthy people, individuals who performed the task in <20 seconds were more likely to be independent in transfers and the time taken to perform the task increased with age[317].

The TGUG is a useful tool for assessing response to intervention such as response to change in rehabilitation programs in elderly orthopaedic patients[318]. It has also been used to assess changes in functional status in pharmacological interventions[196].

The TGUG test is a simple, inexpensive test which is a useful measure basic mobility in older people and can be easily used in a clinical setting. For the purposes of this thesis the TGUG test was used to assess changes in functional status with spironolactone intervention in older people.

The Incremental Shuttle walk test (ISWT)

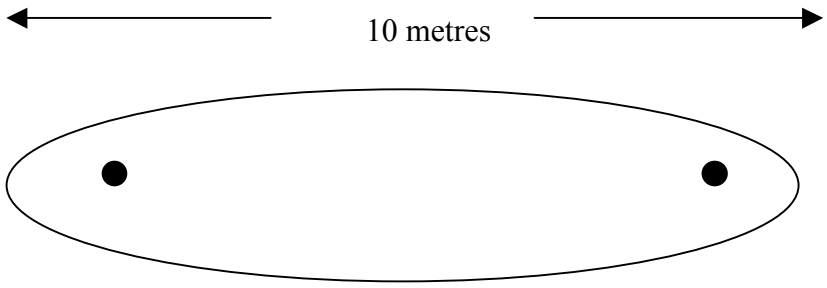
The ISWT is a set paced walking test which is a useful method to measure exercise capacity. The ISWT was carried out along a quiet corridor free from obstacles and distractions. Participants walked up and down a 10 metre course (1 shuttle). The course was identified by two cones inset 0.5 metre from either end of the 10 metre course (Figure 2.4). The speed at which the participant walked was dictated by a pre-recorded audio signal (bleep), played on a compact disc (CD) player. Standardised instructions for the test were pre-recorded on the CD and played to each participant prior to commencing the test. Participants were advised to stand at the first cone in preparation for the beginning of the test.

The start of the test was indicated on the CD by a triple bleep. Thereafter the CD emitted a single bleep at regular intervals, at which point the participant should have turned around the cone in an attempt to walk back down the course. The speed of the walking increased in increments (0.17m/s) every minute. Participants were advised to continue to walk until they could not maintain the speed or until they became too tired, breathless or experienced any discomfort. The total number of completed shuttles and total time taken in seconds to complete the task were recorded.

Field walking tests are often used as objective measures of disability. However, the majority are self-paced tests when patients may not reach their maximal aerobic exercise capacity, depending on how the test is conducted. Self-paced tests are also influenced by factors such as encouragement and are therefore more difficult to standardise[294].

The ISWT was initially developed by Singh and colleagues in 1992 in order to overcome some of the shortfalls of self-paced walk tests. Singh demonstrated that as an externally paced field walk test, the ISWT incorporated an incremental and progressive structure, stressing patients to a symptom limited maximal performance[297]. An association was also noted in the ISWT between a graded increase in work rate and an increase in cardiovascular response as shown with an increase in heart rate, which was not observed in previous self-paced walk tests. Unlike treadmill or cycle ergometry, this test is based on walking, a familiar daily activity. As a standardised test the ISWT also allows valid comparisons both within and between participants. Participants who complete the same distance in the ISWT experience the same work rate compared to self-paced walk tests.

Figure 2.4 – Diagram of the shuttle walk test (Singh *et al*)[297]



The original study by Singh and colleagues demonstrated a close association between distances covered in the ISWT and the distance covered in the 6MWT. They also noted that the ISWT was reproducible after only one practice [297]. Compared to the 6MWT in patients with COPD, the shuttle walk test appears to be more responsive to change in clinical condition[319]. However, the main disadvantage of the ISWT is its lack of validity in older people.

Reproducibility of the ISWT has been demonstrated not only in patients with COPD but also in other patient populations. In 10 patients with dual chamber pacemakers the ISWT was repeated on three separate occasions. There was no significant difference in the duration of the tests or relative cardiac outputs generated during the three consecutive tests[320]. This suggests no learning effect and good reproducibility. The study also showed good responsiveness to change with exercise duration and peak relative cardiac output significantly different between patients with different dual chamber pace makers.

Performance during the ISWT has shown to be predictive of maximum oxygen uptake (VO_2) during laboratory treadmill testing in patients with ischaemic heart disease[321], heart failure[322] or chronic pulmonary disorders[323].

The ISWT is a useful measure of response to treatment in pulmonary rehabilitation programs in patients with COPD. Singh *et al* showed minimal clinically significant improvement for the ISWT in patients with COPD following pulmonary rehabilitation of 47.5 metres. Patients were also able to distinguish an additional benefit if the change in distance walked was greater than 78.7 metres[324]. This demonstrates the use of the ISWT as a measure of exercise capacity following intervention. However, it is worthwhile noting that the distance walked in older people is more likely to be limited by lower limb tiredness or weakness rather than breathlessness; therefore results may vary in an older population.

The feasibility and reproducibility of the ISWT was analysed in a cohort of 82 non-institutionalised older adults (>70 years), 50 of whom suffered from COPD. The study

showed that, although there was no association between the ISWT distance and age, the test was feasible and reproducible in an older population. 88% of subjects with COPD completed the test. A correlation was found between the ISWT distance walked and measures of activities of daily living ($r=0.51$)[325]. This could suggest a potential role of the ISWT as a marker for disability in this age group. However, results from this study should be treated with caution as most subjects were selected on the basis they were independent in self-care therefore, further research is required for its use in frail older people. More importantly the study showed that the ISWT was sensitive to change following the administration of bronchodilators. This demonstrates that the ISWT could be an appropriate measure of response to change in interventional studies in an older population.

Peripheral muscle mass and strength has been shown to relate to both laboratory cycling and self-paced walk tests. More recently, a study involving 85 stable COPD patients (mean age= 67 years, SD= 9 years) showed that improvement in ISWT performance from strength training closely correlated with quadriceps muscle strength but not muscle mass[326]. This suggests that muscle strength is important for maximal exercise capacity as measured by the ISWT and that muscle quality, but not muscle quantity, has a greater influence on peak walking performance. It was also suggested that reductions in exercise capacity in COPD patients may have reciprocal effects on muscle strength because of muscle disease and de-conditioning. Therefore, a similar effect might also be expected in older sedentary adults. It also demonstrates the potential use of the ISWT in interventional studies as it measures not only functional but physiological changes in individuals.

The endurance shuttle walk test (ESWT) was later developed in order to measure a person's ability to sustain sub-maximal exercise which is necessary to perform daily activities[327]. In a cohort of patients with COPD the ESWT showed good repeatability after one practice walk and was more sensitive to change following a 7 week pulmonary rehabilitation program that the ISWT. However, the test does require patients to do a warm-up practice test for approximately 100 seconds prior to the start of the test. This addition could potentially exclude very frail or disabled patients from being assessed[327]. Therefore, for the purposes of this study it was concluded that the ESWT would be an inappropriate test to perform as it would exclude very frail or functionally impaired older people whom we are seeking to assess.

In this thesis the ISWT was used to measure change in maximal exercise capacity (peak VO_2) with spironolactone in older people. It has good reproducibility and is responsive to change. It can also be carried out in a smaller space compared to 6MWT making it useful in a busy clinical setting.

EuroQol questionnaire (EQ–5D and Visual Analogue Scale)

The aim of the EuroQol questionnaire is to assess overall wellbeing and health status in addition to functional status by using a single index score. It is divided into two sections the EuroQol EQ-5D utility section and the Visual Analogue Scale (EQ-VAS). The EQ-5D is a brief questionnaire with a self-reported description section, consisting of five domains (mobility, self care, usual activities, pain and discomfort and anxiety and depression). Each of the domains has a three point categorical response scale associated with 'health today'. Participants are asked to choose one of the three descriptive statements regarding their health from level 1 "no problem", level 2 "some or moderate problems" or level 3 "extreme problems". All 5 responses are recorded as a 5-digit code giving a total of 243 possible EuroQuol health states. This was then converted to a single index quality of life score ranging from 0 to 1.

The EQ-VAS incorporates a 20 cm vertical visual scale which represents overall health status. The scale ranges from 0 (worst imaginable health state) to 100 (best imaginable health state). Participants were asked to draw a line from the box marked 'your own health state today' to the appropriate point along the visual analogue scale to indicate where they thought their overall health state was on that day.

The EQ-5D questionnaire was first developed in 1987 by the EuroQuol group as a standardised non-disease-specific instrument for assessing quality of life[328]. It was purposefully developed as a generic measure of quality of life that has been extensively validated as a reliable test in both the general population and in other patient groups[328-330]. The advantage of using this quality of life questionnaire is that as well as being simple to use, it can be used to compare measures of quality of life between people of

different age groups and between people with different co-morbidities. These comparators are particularly relevant to an older population.

With only five domains to address it is considered to be simple to complete especially in an elderly population. The EQ-5D has been widely used as a measure of health status in older people and has been demonstrated to be both valid and reliable in measuring health status. In particular, it has been useful in assessing patients in which a substantial change in health status is expected, such as in patients following surgical repair of hip fracture. Consequently, it is a valuable tool when assessing response to intervention[331].

Other studies found that both EQ-5D and EQ-VAS are sensitive to self-reported changes in disease severity in chronic diseases, such as rheumatoid arthritis or multiple sclerosis[330;332]. There has been conflicting evidence for the use of the EQ-5D and EQ-VAS in measuring health status. In patients with ankylosing spondolytis the EQ-VAS was found to be more responsive to change than the EQ-5D[333]. In a further study involving a cohort of patients with inflammatory bowel disease, the EQ-VAS was more responsive than EQ-5D in measuring deterioration in health. However, EQ-5D was more responsive to improvement in health state in patients with active disease[334] while in patients with Parkinson's disease the EQ-5D closely correlated with disease severity than the EQ-VAS[335]. There is now evidence to suggest that EQ-5D more closely correlates to functional impairment than EQ-VAS[330;336]. The EQ-5D also correlates well to people's perception of their disabilities (Barthel Index, Health Assessment Questionnaires and Modified Rankin Scale). In this case EQ-5D appears to be a valid tool for assessing health related quality of life and in particular impairment in mobility, while EQ-VAS is therefore a useful in measuring overall health and wellbeing. This

makes the EuroQuol questionnaire a useful measure for both functional impairment and a measure of overall health status in our study involving older people.

Functional Limitation Profile (FLP)

The Functional Limitation Profile (FLP) is a useful tool in measuring health status by assessing the impact of illness on daily activities and behaviour.

The FLP is a UK modified version of the Sickness Impact Profile (SIP) used to assess health status in individuals with both acute and chronic illnesses[337]. The FLP is regarded as a good measure of functional status when assessing functional impairment covering a minimum to maximum degree of functional impairment while being sensitive to small changes within the range of limitation[338].

The FLP covers 136 items grouped into 13 different categories. Each category relates to a different aspect of daily functioning: Physical (ambulation, mobility, body care, and movement) social (social interaction, communication, emotional behaviour, alertness and behaviour) and the remaining categories (eating, work, sleep, household management and recreation and pastimes). Each item is given a score which reflected the relative severity of limitation associated with the item. For the 13 different categories there is a list of 136 statements. The participants are instructed to listen to each of the descriptions as read by the supervisor. They are required to choose only one statement per category that best describes them emotionally, physically and socially on that day. Each description within the category is weighted on a scoring system going from severe impairment to less severe impairments physically, emotionally or socially.

If the participant is unable to choose a suitable description it was documented as no response. Once all 13 categories are completed scores are added and two sub-scores were given for physical domain (total score = 454) and psychosocial domain (total score = 567). All domains are then added together to give a grand total score (total score = 1652). The higher the score, the more functionally impaired the individual.

The aim of the FLP/SIP is to assess the impact of illness on behaviour[339]. The items in the profile cover three major aspects of health as recommended by the World Health Organization including physical, mental and social. Developers of the SIP / FLP conceptualized illness as "changes in behaviour associated with carrying out an individual's activities of daily living"[337]. It is intended to provide a measure of health status in individuals and populations with chronic and acute illness and to measure illness-related functional limitation, while remaining sensitive to change.

In the original study involving the development of the SIP, a weak correlation between category score indicated minimal overlap between categories so recognising each category as an important entity of the whole instrument. Bergner *et al* also reported consistently high test-retest reliabilities for the overall SIP score[340]. The SIP correlates closely with other generic measures of health status such as the Short Form Questionnaire (SF-36) in physical, emotional and overall functioning in elderly male veterans[341]. The Nottingham Health Profile (NHP) is another commonly used measure of health-related behaviour. Although the NHP has shown sensitivity to change between different types of patient groups and is responsive to major treatment effects such as heart transplant and coronary bypass surgery there is insufficient evidence for its responsiveness to less dramatic interventions. Unlike the NHP, the SIP is more responsive to treatment effects in interventional studies and is acceptable to complete,

with little evidence to suggest problems with compliance in an older population[342].The main disadvantage of the questionnaire is primarily the length of time it takes to complete which, on averages takes between 20-40 minutes. Some researchers have suggested that the SIP/FLP should be shortened by deleting items which are not considered relevant to their specific population. There is debate whether this will jeopardise the validity of the category as well as the overall score. Although abbreviated versions have been used in patients with rheumatoid arthritis and nursing home patients there is a lack of evidence to suggest their use in a wider older population[343].

The SIP has shown to be a useful measure of physical functioning and has been used in interventional studies to monitor change in functional state. In one study endurance exercise training improved exercise capacity as well as improving quality of life and functional state, in a cohort of older women with heart failure suggesting a correlation between exercise capacity and functional state as measured by in the physical category of SIP/FLP[344]. In a longitudinal study involving patients with rheumatoid arthritis, changes in the physical domain of the SIP corresponded more closely to changes in patients' clinical findings than changes in the psycho-social domain of the profile[345].

Overall the SIP/FLP is a valid and reliable measure of general as well as functional status. It also demonstrated good correlation to other functional status measures and is acceptable to use in an older population[346]. This makes the FLP a useful measure of baseline health status. In this thesis we will also use the FLP to monitor functional state in response to intervention with spironolactone in an older population.

The Hospital Anxiety and Depression Scale (HADS)

The HADS is a self report scaling system of fourteen items on a 4-point scale (range 0-3). It is divided into an anxiety sub-scale (HADS-A) and a depression sub-scale (HADS-D). Each sub-scale incorporates seven of the total fourteen items intermingled in the questionnaire. Each of the 14 items related to symptoms of anxiety or depression. The participant is then instructed to choose only one of four answers rated on the 4-point scale, in relation to their general mood over the preceding month. After all 14 items are completed a total score for each sub-scale is calculated form the total of its respective 7 items (total of each sub-scale range 0-21).

The HADS was initially developed as a screening tool for identifying and quantifying severity in patients with anxiety and depression. However, it was later felt that as a diagnostic tool it failed to incorporate some of the somatic symptoms of depression. The lack of measurement of somatic symptoms proves useful as a measure of psychological status as it excludes the influence of chronic disease on physical state.

Epidemiological studies have shown that both anxiety and depression are prevalent in an elderly population with up to 12% of the community dwelling elderly population in Europe suffering from depression and up 27% in older institutionalised patients[347;348]. The prevalence of anxiety disorders in community dwelling older people has been estimated to be 15%[349]. Depression can also contribute to a decline in health state and is a poor prognostic indicator in many chronic diseases such as diabetes mellitus[350], renal failure[351], and heart failure[352].

Depression is a strong predictor of mortality as well as deterioration in functional state. Patients with depressive symptoms had an 82% higher risk of either functional decline

or death[353]. Older people with persistently elevated depressive symptoms experience a steep functional decline when compared to non-depressed or temporarily depressed older people[354]. Anxiety has also been associated with worse exercise performance in patients with emphysema[355]. When assessing changes in functional state it is therefore important to measure symptoms of anxiety and depression.

The HADS provides a valid and reliable screening tool for depression and anxiety. It has been suggested that there are two cut-off sub-scale scores for detecting anxiety or depression 8 to 10 = borderline cases requiring follow up assessment while, valid cases requiring further management score >11[356].

The HADS questionnaire correlates well with other questionnaires for anxiety and depression such as the Beck Depression Inventory (BDI)[357] and the State Trait anxiety score (STAI)[358]**.** In a systematic review of 747 papers that used HADS scale, the sensitivity and specificity of HADS-A and HADS-D range from 0.7 to 0.9 with a threshold of each subscale >8.

Most other rating scales for anxiety and depression were developed for use in psychiatric populations and invariably included somatic symptoms. The Hamilton Depression scale is one of the most popular scales for detecting depression. However, it contains a number of items which relate to somatic symptoms of depression. In older people there is often an overlap between the somatic symptoms of anxiety and depression and symptoms of chronic disease. It could therefore be speculated that depression could be overestimated in an older population using these scales. The Geriatric Depression Scale (GDS) has been developed for use in older adults and has the

advantage of avoiding the use of somatic symptoms. However, the validity of the GDS appears to be dependent on the degree of cognitive impairment, as subjects with cognitive impairment were more likely to be unable to complete the questionnaire[359]. In contrast, HADS has shown good validity to screen depressive symptoms in older people in comparison to GDS[359]. The lack of measurement of somatic symptoms in the questionnaire avoids an overlap between physical and psychological health. The simple structure of the HADS has also made it a useful screening tool for depression in patients with intellectual disabilities[360].

Anxiety and depression have been shown to have a negative effect on quality of life in older people[361]. With a growing number of clinical trials involving an older research population, the prevalence of anxiety and depression in this age group could impact on clinical outcomes of randomized controlled trials. As part of a study to determine the cost benefits of influenza vaccine in fit, healthy older people aged 65-74 years of age, the participant's symptoms of anxiety and depression were monitored using HADS, quality of life measured by the EQ-5D and functional ability was measured by the Barthel Index. The study showed that although the prevalence of anxiety and depression was low for this age group, individuals with anxiety or depression (HADS>8) were more likely to suffer from perceived systemic side effects after influenza vaccination[361]. The EQ-5D quality of life score and the Barthel Index were also significantly reduced in patients with both anxiety and depression. Therefore, when measuring outcomes such as quality of life and functional ability in older people care must be taken to ensure that anxiety and depression does not affect results more than the intervention.

HADS is a useful tool for measuring psychological status in older people and has been widely used in clinical settings. For the purpose of this thesis the HADS questionnaire was chosen as a measure of psychological state in older people.

Other Measures

Urea and electrolytes including serum magnesium

A 3mL sample of venous blood was taken from each participant in a gold vacutainer for analysis of renal function (serum sodium, potassium, urea and creatinine) and serum magnesium levels. Samples were delivered to the Biochemistry Department at Ninewells Hospital, Dundee. Samples were taken at weeks 0, 2,5,10 and 20 to monitor changes in renal function with spironolactone treatment. Results of urea and electrolytes, including serum magnesium, were checked by LB the following day and results were recorded. Serum magnesium was measured to monitor changes in the RAAS. As well as increasing sodium re-absorption from the distal and collecting tubules, aldosterone also promotes excretion of magnesium and potassium from the body. Magnesium is involved in muscle ATP production which facilitates muscle contractility and function. In this thesis, serum magnesium was measured to assess changes in the RAAS and to asses if there was a relationship between serum magnesium and functional state in older people without heart failure.

Plasma Aldosterone

Venous blood samples for aldosterone were taken at baseline and 20 weeks to measure changes in the RAAS. A 5 ml sample of venous blood was taken in a green Lithium heparin vacutainer. The sample was kept on ice and then centrifuged as soon as possible for 10 minutes at 3000rpm at 4^0C. Plasma was extracted from the centrifuged sample and placed in 1ml sample tube with a minimum requirement of 0.5 mls. The sample was then transferred to a -20^0C freezer and stored until the last participant has completed their final visit, at which point the samples were assayed. At the end of the study, Aldosterone was measured using the ALDOCTK-2 kit (DiaSorin Inc, US).

Aldosterone is a key regulatory hormone for electrolytes. It binds to mineralocorticoid receptors (MR) within the epithelial cells of the distal convoluted tubules of the kidney nephron, promoting sodium re-absorption and potassium excretion and binds to MRs in the non-epithelial cells (e.g. heart, blood vessels and brain). Spironolactone acts as a competitive antagonist, blocking the binding of aldosterone to the MR and interferes with sodium/potassium exchange, reducing urinary potassium excretion and increasing serum potassium levels. Spironolactone has shown to produce a sustained increase in plasma aldosterone, consistent with inhibition of the negative regulatory feedback of RAAS. However the increase in plasma aldosterone does not overcome the effects of spironolactone.

In this thesis plasma aldosterone was chosen to monitor the inhibition of the RAAS with spironolactone and whether there was a relation between aldosterone blockade and physical function in older people without heart failure.

Plasma B-type Natriuretic peptide (BNP)

A 7ml sample of venous blood was taken in a purple vacutainer containing potassium EDTA. The sample was kept on ice and then centrifuged as soon as possible for 10 minutes at 3000rpm at 4^0C. A 2ml aliquot of plasma was extracted and placed in a 5ml sample bottle. The sample was then transferred to a -70^0C freezer and stored until the last participant has completed their final visit at which point the samples were assayed. At the end of the study the BNP was assayed with the Bachem radioimmune assay kit (Bachem, Peninsula Laboratories, Inc, US).

Atrial natriuretic peptide (ANP) is synthesised in the atrium and is released in response to atrial stretch. This peptide plays an important part in sodium and water homeostasis. ANP is also involved in cardiovascular function where it is elevated in congestive cardiac failure[362]. In contrast, BNP is released from the cardiac ventricles. Levels are significantly elevated in overload states such as congestive cardiac failure. BNP correlates better than ANP to severity of congestive cardiac failure as well as mortality in patients with chronic congestive heart failure[363].

Natriuretic peptides have a range of physiological actions including induction of natriuesis and diuresis, vasodilatation, inhibition of cardiac remodelling, inhibition of sympathetic outflow and inhibition of RAAS[362].

More recently, elevated BNP levels have been associated with a wide range of other cardiovascular diseases including atrial fibrillation[364], left ventricular hypertrophy[194] and coronary heart disease[365].

Plasma BNP levels rise with age. In a cohort of subjects aged 55-64 years plasma BNP levels were 26±2 pg/ml. This level increased to 31±2 pg/ml and 64±6 pg/ml in subjects aged 65-74 years and >75 years respectively[366]. In a group of functionally impaired older people an elevated plasma BNP level was strongly associated with all-cause and cardiovascular mortality even without a prior cardiovascular event[367].

Studies have shown a correlation between BNP and functional capacity as measured by the New York Heart Association (NYHA) classification and exercise tolerance (measured by 6MWT). An increasing level of plasma BNP has been associated with a worse performance in the 6MWT[368]. Elevated BNP levels have been associated with impairment in maximum exercise performance ($V0_2$) and also differentiated between chronic heart failure patients with moderately and severely impaired exercise performance[369]. Individuals with a raised plasma BNP level are more likely to be limited in daily physical activities[370]. It is suggested that BNP should be used as a surrogate bio-marker to assess functional capacity in patients with heart failure. Patients with chronic COPD tend to have a rise in plasma BNP due to increased right-sided myocardial stress as a result of pulmonary hypertension. This rise in plasma BNP significantly correlated to a rise in one-year mortality in COPD patients with normal systolic function[371].

BNP may have a role in assessing efficacy in pharmacological interventions as well as associated changes in functional status in patients with heart failure, either through an improvement in exercise tolerance or functional status by improvement in NYHA class[372]. A correlation has been shown between improvement in exercise duration and a

fall in plasma BNP via ACE inhibition[373]. The correlation between BNP levels and exercise tolerance with ACE inhibition was shown to be dose related[374]. It could be suggested that similar effects may be seen with other heart failure therapies such as spironolactone and angiotensin II receptor blockers.

In this thesis, BNP levels were measured to assess the relationship between BNP and physical function and whether the use of spironolactone would reduce BNP levels in older people without heart failure.

Plasma cortisol

A 5 ml sample of venous blood was taken in a green Lithium heparin vacutainer. The sample was kept on ice and then centrifuged as soon as possible for 10 minutes at 3000 rpm at 4^0C. Plasma was extracted from the centrifuged sample and placed in 1ml sample tube with a minimum plasma volume of 20 uL required for analysis . The sample was then transferred to a -20^0C freezer and stored until the last participant has completed their final visit, at which point the samples were assayed. At the end of the study plasma cortisol levels were analysed measured using a radioimmunoassay (supplied by Diasorin, UK) and the inter-assay coefficients of variance were 8.6%.

The actions of cortisol are mainly triggered through the glucocorticoid receptors in the tissues; however cortisol has a similar affinity for the MR as aldosterone. Levels of glucocorticoids are around 100 times higher than mineralocorticoids in the circulation. To allow the selective mineralocorticoid action the enzyme 11-β HSD exists in the mineralocorticoid target tissues and catalyses the deactivation of glucocorticoids.

Basal cortisol levels increase with age. Cortisol is known to cause inhibit the synthesis of muscle proteins[375] and conditions of hypercortisolism are associated with muscle weakness and atrophy. More recently Peeters *et al* found a negative association between high cortisol levels and physical performance in older women[376]. Studies have shown that mineralocorticoid antagonism with spironolactone, can lead to a negative feedback on the hypothalamic-pituitary axis (HPA), resulting in a subsequent rise in ACTH and cortisol. In this thesis plasma cortisol levels were measured to evaluate the relationship between cortisol levels and physical function in older people and to assess the effects of spironolactone on the HPA axis.

2.7 Power and sample size calculation

The minimum clinically important difference in distance walked in 6MWT is 30 metres[286]. Based on results from a previous study in our department involving functionally impaired older people without heart failure, the anticipated mean 6MWT distance was 300 metres, with a standard deviation for change in 6MWT distance of 50 metres[196].

A power calculation estimated that a sample size of 88 participants (44 participants per group) was predicted to have 80% power to detect a 30 metre difference in the distance walked in the 6MWT between groups at p=0.05 significance level, using a two-sided test.

Trials involving older people will inevitably have people who drop out due to either illness or death. A previous study in this target population in our department showed a drop out rate of 27% at 20 weeks[196]. In anticipation of a drop out rate of 27% a projected sample size of 120 participants was required in order to yield 44 completing participants per group.

2.8 Data entry and management

Data from study visits were recorded for each participant on a CRF by LB. Other data recorded included log sheets for concomitant medication, adverse events and serious adverse events.

Data entry and management were performed independently by the Robertson Centre for Biostatistics (RCB) at the University of Glasgow. The centre is part of UK Clinical Research Network (UKCRN) registered Glasgow Clinical Trials Unit. All RCB studies are managed to the highest possible standards in accordance with the International Conference on Harmonisation good clinical practice guidelines. The RCB has extensive experience of managing data in the context of privacy and data protection legislation, including the Data Protection Act of 1998 and the European Union Data Protection Directive 95/46/EC. As a result, extensive data security systems and procedures are in place including firewall protected networking facilities and secure data transfer protocols. It is audited every six months by the British Standards in Industry and has also been subject to rigorous external audits from Medicine and Healthcare Products Regulatory Authorities (MHRA).

For this study, RCB was involved in CRF design, data processing (data entry and verification) and data management (including data querying and validation). In addition, RCB provided a system for reporting serious adverse events to Pharmacovigilance and to other authorities such as MHRA when relevant.

All completed CRF's were checked by LB, and sent to RCB by courier. All entered data was then transferred back to LB on Microsoft Excel comma separated variable files.

Data was then transferred from Microsoft Excel database to Statistical Package for Social Sciences (SPSS) software version 18.0 for statistical sub-group analysis.

2.9 Statistical Analysis

Data were entered onto a database by RCB and analysed using SAS statistical package, (version 9.2). Analyses were performed only after all data were entered and the database had been locked. The allocation of treatment code was obtained (as group A or B) from Tayside Pharmaceuticals after the database had been locked. Analyses of the primary and secondary outcomes were performed prior to breaking the treatment codes. Subgroup analyses were performed by LB using SPSS statistical package (version 18.0), after treatment codes were broken.

Using intention-to-treat analysis, differences between treatment groups for outcome measures were assessed using Student's t-test. Analyses of covariance (ANCOVA) models were used to compare the change in outcomes across the treatment groups at 10 and 20 weeks. To compensate for effect of regression to the mean, the change in outcome measures from baseline were analysed using ANCOVA models, using baseline outcome measures and age as covariates. As a safeguard against any data missing at random, analysis of the primary outcome was performed using multiple imputation.

3. RESULTS

3.1 Recruitment

Participants were recruited from the Medicine for the Elderly services in NHS Tayside and from 6 GP practices within Primary care, using the East of Scotland node of the Scottish Primary Care Research Network (SPCRN). Recruitment took place over an 18 month period from December 2008 and June 2010.

A total of 3398 potential participants (2626 from Primary Care and 772 from Medicine for the Elderly) had a preliminary screening assessment of eligibility. Initial screening was carried out by checking the hospital medical case notes of those attending the Medicine for the Elderly services or by checking the electronic patient database of those from Primary Care. Of these 571/3398 (17%) from Medicine for the Elderly were found to be ineligible. The remaining 2827 were contacted by letter, of these 2626 letters were sent to older people in Primary Care and 201 letters were sent to older people attending the Medicine for the Elderly services. From the total number of people who were contacted by letter 87% (2460/2827) failed to reply. A higher proportion of older people attending the Medicine for the Elderly service failed to reply (185/201) compared to those in Primary Care (2275/2626).

All 367 potential participants (351 from Primary Care and 16 from Medicine for the Elderly service) who replied were contacted by telephone and screened further to assess eligibility. Of these, 130 participants were invited to attend a detailed screening visit at either the Royal Victoria Hospital in Dundee or Perth Royal Infirmary from which 120 participants were recruited to the study. Figure 3.1 shows the flow of participants

through the study and the reasons for failing the hospital screening visit. The majority of participants recruited were from Primary Care (106/120) and only 12% (14/120) were recruited from the Medicine for the Elderly services.

Despite using the GP practice electronic patient database to exclude potential participants already taking medications that would affect the renin-angiotensin-aldosterone system, 7% (26/351) of those from Primary Care were found to be already taking either an ACE inhibitor or Angiotensin receptor antagonist. A further 1% (4/351) was already taking spironolactone. Those who were found to be already taking either an ACE inhibitor of Angiotensin receptor antagonist, came from the same GP practice, where the Practice Manager carried out the database search instead of the SPCRN research co-coordinator. Of the 4 individuals who were prescribed spironolactone, 3/4 had recently started on the tablet by their GP for newly diagnosed heart failure and 1/4 was prescribed the drug at a hospital outpatient clinic but the GP had not been made aware of the change in medication.

Almost half (180/351) of the people who replied from Primary Care did not meet inclusion criteria on telephone screening as they reported no problems with activities of daily living. As the electronic patient databases used in Primary Care do not hold information regarding patients' functional state, letters were sent to people in Primary Care with a wide range of physical abilities. Their baseline functional state was established only after telephone screening. In contrast, patients who attended the Medicine for the Elderly services usually had a full functional assessment carried out which was documented in their hospital case notes. Table 3.1 summarises the reasons for people not meeting the inclusion criteria.

Figure 3.1. CONSORT Diagram

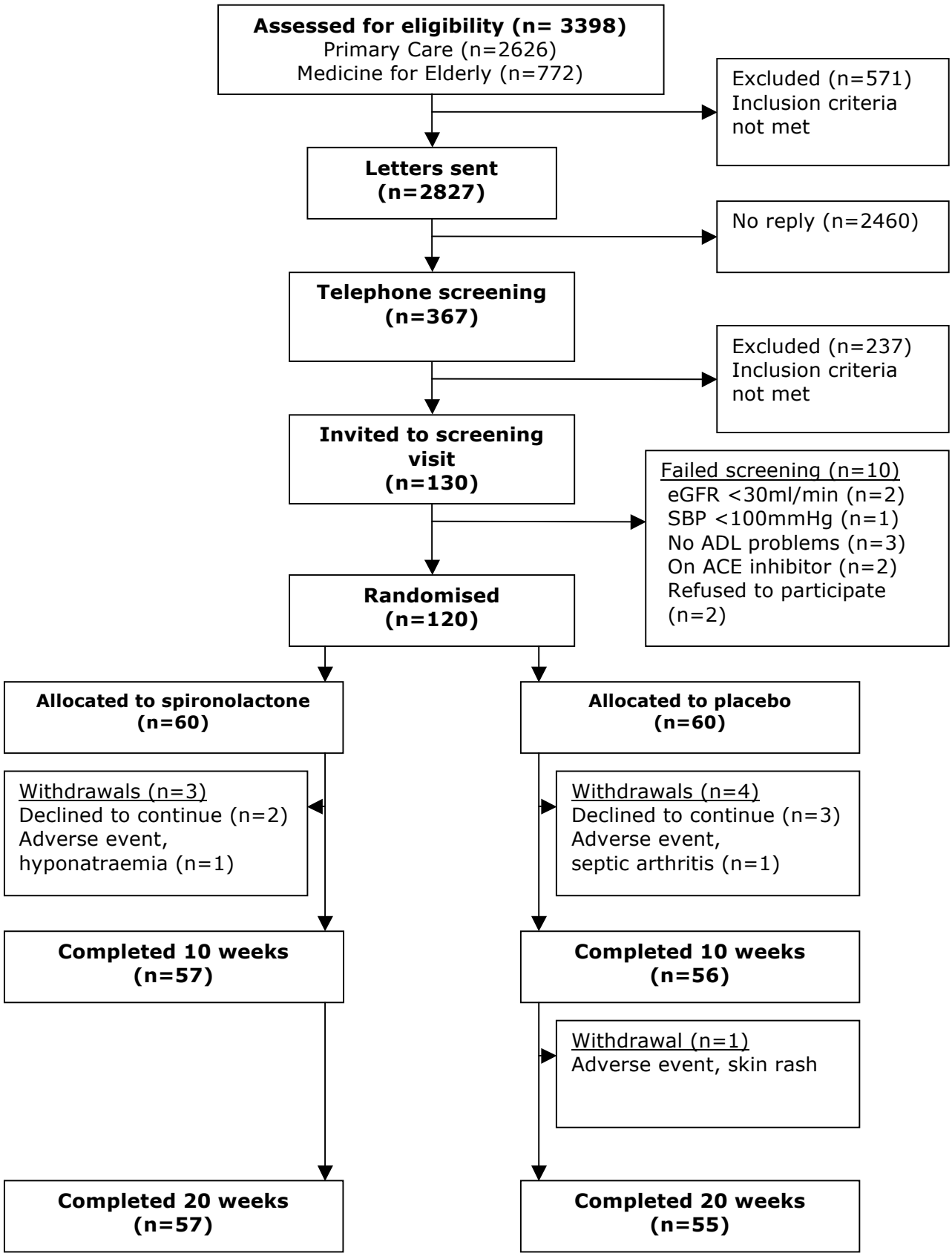


Table 3.1 Reasons for participants not meeting study inclusion criteria

Reasons for non-eligibility	Medicine for the Elderly Services (n=571)	Primary Care (n=237)	Overall number (n=808)
On ACE inhibitor or ARB	158 (27.8%)	26 (10.9%)	184 (22.7%)
On spironolactone	32 (5.6%)	4 (1.7%)	36 (4.5%)
MMSE < 15	57 (10.0%)	0	57 (7.1%)
eGFR < 30ml/min	42 (5.2%)	6 (2.5%)	48 (5.9%)
Presence of LV systolic dysfunction	85 (11.9%)	4 (1.7%)	89 (11.0%)
Previous history of orthostatic hypotension	46 (8.1%)	5 (2.1%)	51 (6.3%)
Nursing Home resident	37 (6.4%)	0	37 (4.6%)
Wheelchair bound	28 (4.9%)	2 (0.8%)	30 (3.7%)
In another trial	4 (0.7%)	0	4 (0.5%)
No problems with activities of daily living	72 (12.6%)	180 (75.9%)	252 (31.2%)
Intolerance to spironolactone	5 (0.9%)	0	5 (0.6%)

3.2 Baseline characteristics

Of the 120 patients randomised 54% (65/120) were male. Participants had a mean (SD) age of 75 years (6) with 27% aged 80 years or over. Sixty five percent (90/120) were prescribed >4 medications with 13% (16/120) prescribed >10 medications. Although 19% (23/120) of participants were diagnosed with COPD only 35% (8/23) were prescribed regular inhaled steroids. This may suggest that participants with COPD were either well controlled or were diagnosed with COPD but were not symptomatic. Table 3.2 shows baseline characteristics of the participants.

Participants were generally well matched between treatment groups. Of the participants in the spironolactone group 37% (22/60) required a walking aid compared to 23% (14/60) in the placebo group. A significant proportion of participants in the spironolactone group were found to have Parkinson's disease (6/60) compared to the placebo group (1/60) (p=0.05). For all other co-morbidities the participants within each of the two treatment groups were well matched.

The mean baseline systolic blood pressure was 149mmHg (SD 20) and 65% (79/120) of participants had a baseline systolic blood pressure of >140mmHg of which only 9% (7/79) were already prescribed antihypertensive therapy. Participants in the spironolactone group were found to have a slightly higher systolic and diastolic blood pressure at baseline, with a between group mean difference of 4 (95% CI -3 to 12) mmHg and 3 (95% CI -1 to 7) mmHg respectively. However, this was not statistically significant.

Overall, the baseline renal function was particularly good for this cohort of patients with a mean eGFR of 88 ml/min (SD 17). Participants in the spironolactone and placebo groups were well matched for baseline renal function and serum magnesium. Baseline data for aldosterone and B-type natriuretic peptide hormone level (BNP) were skewed and between group differences were analysed using Mann Whitney tests.

Aldosterone levels were 25% higher in the placebo group compared to the spironolactone group at baseline (p=0.04). In the placebo group 48% (29/60) had baseline aldosterone levels of >100 pg/ml compared to 37% (22/60) in the spironolactone group (p=0.18). Of the participants in the placebo group with a baseline aldosterone >100 pg/ml, 34% (10/29) were known to have hypertension compared to 9% (2/22) of participants in the spironolactone group (p=0.03). Two of the participants in the placebo group with aldosterone levels >100 pg/ml (328.9 pg/mL, 251.6 pg/mL) were taking 2 or more antihypertensive medications for resistant hypertension which could account for the significant difference in aldosterone between groups at baseline. When these individuals were excluded from analysis the difference between the groups in baseline aldosterone levels was not significant (p=0.13).

The overall median BNP was 24.6 (IQR 27.9) pg/ml. Patients over 80 years had a median BNP of 31.9 (IQR 44.9) pg/ml. The median baseline BNP value was similar to values found in other studies involving older people without heart failure[377]. The median BNP in males was slightly higher than in females (26.1 pg/ml vs. 22.2 pg/ml) although not statistically significant (p=0.50).

Table 3.2 Baseline characteristics of participants randomised to spironolactone or placebo.

	Spironolactone (n=60)	**Placebo (n=60)**	***P***
Mean age (yrs) (SD)	75.1 (5.6)	74.2 (6.5)	0.43
Male sex	31 (52.5%)	34 (56.7%)	0.65
Weight (Kg)	78.2 (14.0)	77.0 (18.7)	0.69
Height (cms)	168 (7)	165 (10)	0.15
MMSE†	29 (2)	29 (2)	0.67
Walking aid			
None	37 (62.7%)	46 (76.7%)	0.10
1 stick	17 (28.8%)	11 (18.3%)	0.18
2 sticks	2 (3.4%)	1 (1.7%)	0.18
Zimmer frame	2 (3.4%)	0	0.15
Triwheel walker	1 (1.7%)	2 (3.3%)	0.57
Past Medical History			
Hypertension	19 (32.2%)	16 (26.7%)	0.50
Ischaemic Heart Disease	8 (13.6%)	7 (11.6%)	0.76
Peripheral Vascular Disease	3 (5.1%)	2 (3.3%)	0.63
Myocardial Infarction	2 (3.4%)	1 (1.7%)	0.55
Diabetes Mellitus	5 (8.5%)	7 (11.7%)	0.56
COPD	10 (16.9%)	13 (21.7%)	0.52
Stroke / TIA	5 (8.5%)	4 (6.7%)	0.71
Parkinson's Disease	6 (10.2%)	1 (1.7%)	**0.05**
Osteoarthritis	24 (40.7%)	30 (50.0%)	0.31
SIMD decile			
1-3	20 (33.9%)	22 (36.7%)	0.86
4-6	19 (32.2%)	14 (23.3%)	0.31
7-10	20 (33.9%)	24 (40.0%)	0.42
SIMD (Median, IQR)	5 (4)	6 (5)	

Drug History			
Loop diuretics	3 (5%)	3 (5%)	1.00
Thiazide diuretics	8 (13.3%)	11 (18.3%)	0.43
Aspirin	17 (28.3%)	18 (30.0%)	0.71
Statins	15 (25.0%)	9 (15.0%)	0.17
Calcium channel blockers	10 (16.7%)	6 (10.0%)	0.30
Beta blockers	3 (5.0%)	5 (8.3%)	0.53
Bronchodilators	3 (5.0%)	5 (8.3%)	0.53
Inhaled steroids	4 (2.7%)	4 (2.7%)	1.00
Total no. of medications (median, IQR)	5 (3)	5 (3)	0.97
Systolic BP (mmHg)	152 (22)	147 (18)	0.27
Diastolic BP (mmHg)	83 (9)	80 (10)	0.06
Blood results			
Potassium (mmol/L)	4.3 (0.4)	4.4 (0.4)	0.27
Sodium (mmol/L)	140.1 (2.9)	140.1 (3.0)	0.97
Urea (mmol/L)	5.9 (1.5)	6.2 (1.7)	0.07
Creatinine (umol/L)	73.0 (15.2)	72.7 (13.9)	0.82
eGFR (ml/min) [MDRD formula]	88.4 (17.5)	87.2 (15.8)	0.72
Magnesium (mmol/L)	0.88 (0.1)	0.87 (0.1)	0.93
BNP (pg/ml)†	24.8 (26.8)	24.5 (26.7)	0.30
Aldosterone (pg/ml)†	84 (67)	105 (94)	**0.04**

(Table 3.2 Continued)

Except where mentioned, values are mean (SD).

†Data displayed as median (interquartile range).

Student's t-test for continuous variables and Pearson's chi squared for discrete variables. Data analysed using Mann-Whitney tests for non-parametric variables.

The majority of participants were recruited from Primary Care compared to the Medicine for the Elderly services (88% vs. 12% respectively). Table 3.3 shows the baseline characteristics in each group. Participants were well matched for age, sex, weight, co-morbidities and blood tests at baseline. However, 71% (10/14) of participants recruited from the Medicine for the Elderly department required a walking aid, compared to 25% (26/106) of those recruited from Primary Care ($p<0.01$). Participants from the Medicine for the Elderly department had a greater total number of medications and a lower mean systolic and diastolic blood pressure at baseline compared to those from Primary Care. Although there was a significant difference in cognitive function between groups this may not be of clinical relevance. The groups were well matched for baseline renal function.

Table 3.3 – Baseline characteristics of participants recruited from the Medicine for the Elderly services and participants recruited from Primary Care.

	Participants recruited from Medicine for the Elderly (n=14)	Participants recruited from Primary Care (n=106)	P value
Age	77.2 (5.6)	74.3 (6.0)	0.12
Male (%)	9/14 (64)	56/106 (77)	0.14
Weight (Kg)	73.2 (10.9)	78.1 (16.9)	0.33
Walking aids (%)	10/14 (71)	26/106 (25)	**<0.01**
Past Medical History (%)			
Hypertension	4/14 (29)	32/106 (30)	0.79
Ischaemic Heart Disease	2/14 (14)	13/106 (13)	0.65
Peripheral vascular disease	0/14 (0)	5/106 (5)	0.45
Diabetes Mellitus	2/14 (14)	10/106 (9)	0.42
Parkinson's Disease	5/14 (36)	2/106 (2)	0.45
Osteoarthritis	2/14 (14)	37/106 (35)	0.32
Total number of medications	7 (3)	5 (3)	**0.03**
MMSE†	27 (5)	29 (2)	**0.01**
Baseline blood pressure			
Systolic BP (mmHg)	137.9 (23.2)	150.9 (19.1)	**0.03**
Diastolic BP(mmHg)	75.8 (8.3)	82.4 (9.8)	**0.03**
Blood Results			
Sodium (mmol/L)	139.7 (3.3)	140.1 (2.9)	0.61
Potassium (mmol/L)	4.38 (0.37)	4.37 (0.37)	0.92
Urea (mmol/L)	5.8 (1.5)	6.1 (1.6)	0.61

Creatinine (umol/L)	68.5 (16.1)	73.5 (14.3)	0.86
Magnesium(mmol/L)	0.86 (0.1)	0.89 (0.1)	0.87
Aldosterone (pg/ml)†	74.7 (50.2)	101.1 (83.6)	0.02
BNP (pg/ml)†	28 (64)	24 (24)	0.22

Except where mentioned, values are mean (SD).

†Data displayed as median (interquartile range).

Student's t-test for continuous variables and Pearson's chi squared for discrete variables. Data analysed using Mann-Whitney tests for non-parametric variables.

3.3 Outcome measures

Differences between the two groups for both the primary and secondary outcomes were analysed using Student t-test. Repeated measure regression models were used to compare the two groups after 10 weeks and 20 weeks for both the primary and secondary outcomes. Although most of the main outcome measures were well matched at baseline, several of the secondary outcomes including the EuroQol (EQ-5D) and the Hospital Anxiety and Depression Scale, HADS-D score showed differences between the spironolactone and placebo group. As a result analyses were also carried out adjusting for baseline measures and age to try to reduce the effect of regression to the mean.

Baseline outcome measures

The groups were well matched for baseline 6MWT distance, TGUG and distance walked in ISWT as shown in Table 3.4. The spironolactone group had a marginally higher HADS-D score with a mean between group difference of 1.0 (95% CI 0.05 to 2.0) compared to the placebo group. The EuroQol EQ-5D utility score at baseline was 0.08 (95% CI 0.01 to 0.15) lower in the spironolactone group relative to the placebo group but this was not significant (p=0.07).

Table 3.4 – Mean baseline outcome measures of participants in the spironolactone and placebo groups.

	Spironolactone Mean (SD)	Placebo Mean (SD)	P value
Baseline 6MWT distance (metres)	336 (120)	345 (102)	0.74
Baseline ISWT distance (metres)	220 (131)	241 (139)	0.99
Baseline Timed-Get-Up and Go (seconds)	13.1 (6.2)	13.5 (5.1)	0.69
Baseline Hospital Anxiety and Depressions Scale questionnaire HADS-A score	4.2 (3.4)	4.7 (3.4)	0.87
Baseline Hospital Anxiety and Depression Scale questionnaire HADS-D score	4.8 (3)	3.8 (2.5)	0.06
Baseline EuroQol EQ-5D utility	0.59 (0.2)	0.67 (0.2)	0.07
Baseline EuroQol EQ-VAS (%)	72.7 (16.7)	72.4 (15.3)	0.91
Baseline Functional limitation Profile (FLP) total score	807 (198)	773 (180)	0.34

Data presented as mean (SD) unless stated otherwise.

Table 3.5 shows a comparison of the baseline outcome measures in those recruited from Primary Care compared to those recruited from Medicine for the Elderly department. Results show that participants recruited from the Medicine for the Elderly department were more functionally impaired at baseline compared to those recruited from Primary Care as shown by a higher FLP score. Participants recruited from Medicine for the Elderly walked a shorter 6MWT distance than those from Primary Care at baseline, with a between group difference of -137 (95% CI -210 to -65) metres which was significant ($p<0.001$). Medicine for the Elderly participants took 9.3 seconds longer to perform the TGUG and walked a shorter ISWT distance, with a between group difference of -134 (95% CI -233 to -35), ($p<0.001$) metres compared to the Primary Care participants. They also had a higher baseline HADS-D score than Primary Care participants indicating the presence of depressive symptoms.

Table 3.5 – A comparison of the baseline outcome measures of those recruited from Medicine for the Elderly compared to those recruited from Primary Care.

	Medicine for the Elderly (n=14) Mean (SD)	Primary Care (n=106) Mean (SD)	P value
Baseline 6MWT distance (metres)	218 (112)	356 (103)	**<0.01**
Baseline ISWT distance (metres)	110 (131)	244 (129)	**<0.01**
Baseline Timed-Get-Up and Go (seconds)	21.7 (10)	12.4 (4.1)	**<0.01**
Baseline Hospital Anxiety and Depressions Scale questionnaire HADS-A score	4.8 (4)	4.4 (3)	0.69
Baseline Hospital Anxiety and Depression Scale questionnaire HADS-D score	5.9 (2.2)	4.1 (2.8)	0.03
Baseline EuroQol EQ-5D utility	0.55 (0.22)	0.64 (0.19)	0.14
Baseline EuroQol EQ-VAS (%)	69.6 (15.3)	72.7 (16)	0.53
Baseline Functional limitation Profile (FLP) total score	933 (191)	775 (184)	**<0.01**

Data presented as mean (SD) unless stated otherwise.

Primary outcome – Change in six minute walk distance

The mean change in the 6MWT distance in the spironolactone group increased by 9.1% (30.5 metres) compared to 9.8% (33.7metres) in the placebo group at 20 weeks (p=0.71). However, the observed difference in 6MWT distance was -3.2 metres (95% CI -28.9 to 22.5) in the spironolactone compared to placebo group at 20 weeks which was not significant (p=0.81) (see Figure 3.2). Nor was there a significant difference between groups in distance walked at 10 weeks (Table 3.6).

Of those in the placebo group 73% (40/55) walked further compared to 65% (38/58) in the spironolactone group at 20 weeks. Fifty five percent (15/27) of participants aged >80 walked further after 20 weeks. The changes in 6MWT distance at 10 weeks and 20 weeks were analysed by using different models for adjustment, as shown in Table 3.7. Data were analysed adjusting for baseline 6MWT distance and age as covariates in order to reduce any effect of regression to the mean. After adjusting for these covariates the change in 6MWT distance in the spironolactone group was 3.7 metres (95% CI -28.6 to 21.2) lower compared to placebo at 20 weeks but this was non-significant.

In order to preserve valid statistical inference any missing values which were missing at random were also analysed as a complete data set using multiple imputation. Despite analysing the data set for missing values using the multiple imputation method, there was no significant change in the 6MWT distance between groups at 10 weeks and 20 weeks.

Baseline 6MWT distance has a wide standard deviation this was due to the wide variation in baseline physical function in the study cohort. The 6MWT distance at

baseline correlated to functional state at baseline, measured by the Functional Limitation Profile (FLP) questionnaire. A lower 6MWT distance correlated to an increase in FLP total score (Pearson's r=-0.46, p<0.01). However there was no correlation between the change in 6MWT distance and change in FLP at 20 weeks (Pearson's r= -0.13, p=0.17).

In order to assess whether the variation in participants' baseline physical function influenced the primary outcome analyses were also performed by dividing participants into two groups; those who walked a distance greater than the median 6MWT distance (367 metres) and those who walked less than the median 6MWT distance at baseline (Figure 3.3). In those with a baseline distance greater than the median value the mean change in distance in the spironolactone group at 20 weeks was 44.3 metres (95%CI 11.6 to 76.9) compared to a change of 35.2 metres (95% CI 3.7 to 66.7), (p=0.68) in the placebo group. In those with a baseline distance less than the median value the mean change in distance from baseline in the spironolactone group at 20 weeks was 18.1 metres (95% CI 0.8 to 35.4) compared to 32.1 metres (95% CI 13.5 to 50.6) in the placebo group (p=0.28). This shows that irrespective of baseline 6MWT distance there was no significant change in walking distance with spironolactone.

Table 3.6 – Mean change in 6MWT distance from baseline at 10 weeks and 20 weeks, unadjusted measures.

Outcome measure	Time	Spironolactone (95% CI)	Placebo (95%CI)	Difference between groups [Spironolactone - Placebo] (95% CI)	P value
Change in 6MWT distance (m)	10 weeks	27.4 (11.4 to 43.5)	27.5 (11.3 to 43.8)	-0.1 (-22.9 to 22.7)	0.99
	20 weeks	30.5 (12.5 to 48.5)	33.7 (15.4 to 52.0)	-3.2 (-28.9 to 22.5)	0.81

Table 3.7 – Mean change in 6MWT distance from baseline using models for adjustment.

Outcome measure	Time	Difference between groups [Spironolactone -Placebo] (95% CI)	P value
Change in 6MWT distance (m) Adjusted for baseline distance and age	10 weeks	-1.6 (-23.5 to 20.3)	0.89
	20 weeks	-3.7 (-28.6 to 21.2)	0.77
Change in 6MWT distance (m) Multiple imputation and adjusted for baseline distance and age	10 weeks	-1.8 (-25.2 to 21.6)	0.88
	20 weeks	-6.1 (-29.7 to 17.6)	0.62

Figure 3.2 Mean change in 6MWT distance at 10 and 20 weeks. Vertical lines represent standard deviation.

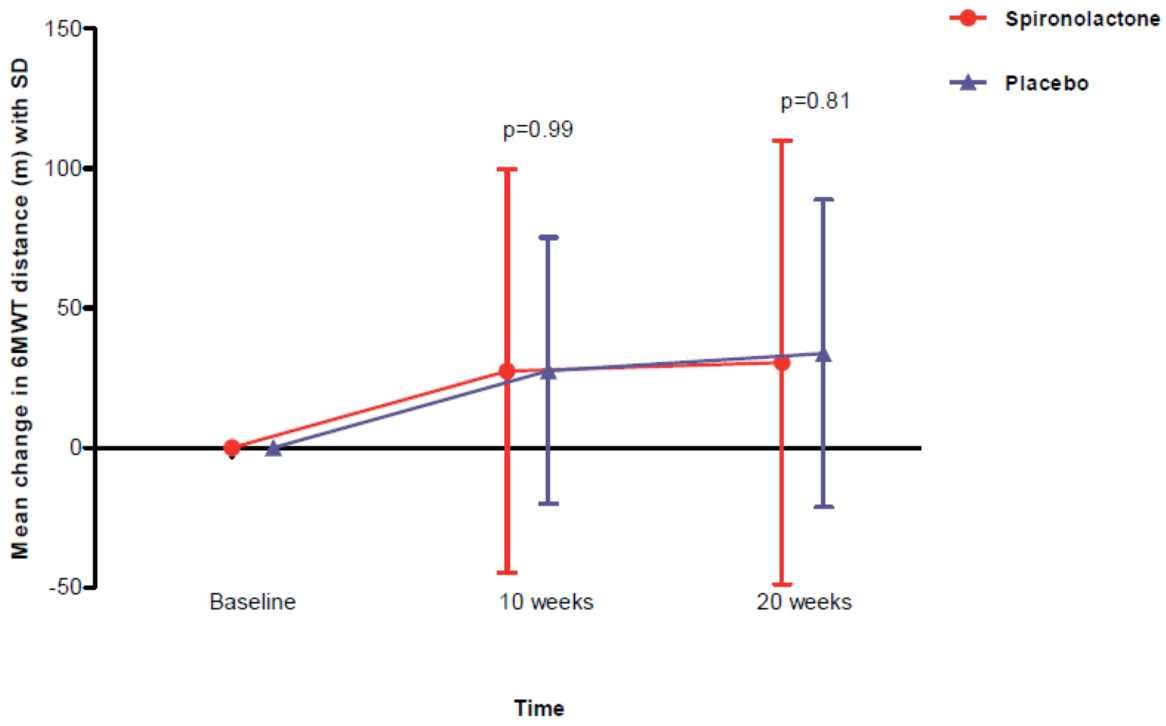
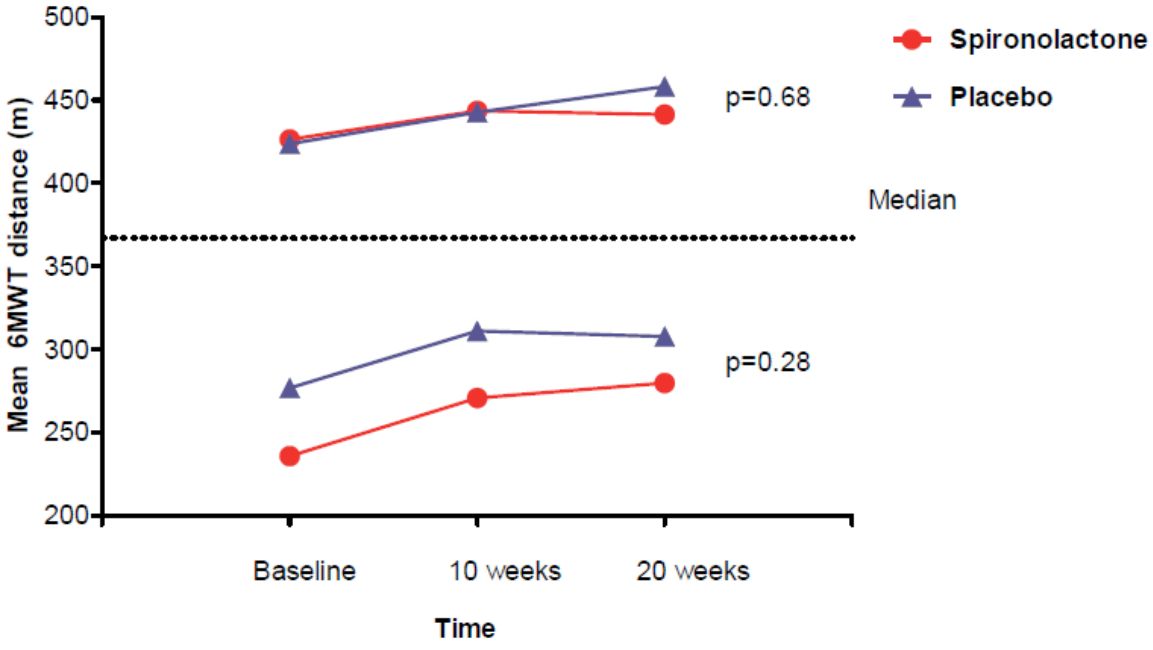


Figure 3.3 - Mean 6MWT distance at baseline, 10 weeks and 20 weeks in individuals who walked above and below the median



The incremental shuttle walk

The mean distance walked in the incremental shuttle walk test (ISWT) at baseline in the spironolactone group was 220 metres (SD131) and 241 metres (SD139) in the placebo group, with no significant difference found between the groups. There were no significant differences between groups as measured by the change in ISWT from baseline at either 10 weeks or 20 weeks. Nor was there any significant change in ISWT between groups after adjusting for baseline ISWT distance and age as covariates, as shown in Table 3.8.

Two participants in the spironolactone group were unable to perform the shuttle walk test at baseline, (n=1) due to joint pain and (n=1) fatigue. This was mainly due to the fact that the incremental shuttle walk test was performed after the 6MWT with a 30 minute rest interval between the tests.

Males walked 59 metres significantly further than females at baseline (p=0.02). Taking this into consideration, we adjusted the change in ISWT distance from baseline, using gender as a covariate. This showed a mean change in the distance walked of 33 metres (95% CI 13.7 to 52.8) in the spironolactone group and 19 metres (95% CI -0.8 to 38.6) in the placebo group at 20 weeks. However, there was no significant change between groups at 20 weeks (p=0.31).

Participants with walking aids had a mean baseline ISWT distance of 122 metres (SD 102) compared to 274 metres (SD 121) in those without walking aids ($p<0.01$). Those with walking aids appeared to find it more difficult to turn around the cones at either ends of the 10-metre course. Adjusting analyses to exclude participants with walking

aids showed a between group change of 7.3 metres (95% CI -24.9 to 39.5) in the spironolactone group at 20 weeks compared to the placebo group but this was not significant (p=0.65).

Table 3.8 Mean change in ISWT distance from baseline, unadjusted and adjusted measures.

Outcome measure	Time	Spironolactone (95% CI)	Placebo (95%CI)	Difference between groups [Spironolactone - Placebo] (95% CI)	P value
Change in ISWT distance (m) Unadjusted	10 weeks	13.9 (-0.42 to 28.3)	17.6 (3.2 to 31.9)	-3.7 (-24.0 to 16.3)	0.72
	20 weeks	25.4 (8.4 to 42.4)	22.4 (4.9 to 39.9)	3.1 (-21.4 to 27.5)	0.81
Change in ISWT distance (m) Adjusted*	10 weeks	13.3 (-1.0 to 27.6)	18.2 (3.9 to 32.5)	-4.9 (-25.2 to 15.4)	0.63
	20 weeks	24.6 (7.6 to 41.6)	23.3 (5.8 to 40.7)	1.4 (-23.0 to 25.8)	0.91

*Adjusted model using baseline ISWT distance and age as covariates

The Timed-Get-Up and Go (TGUG)

The mean time taken to do the Timed-Get-Up and Go (TGUG) in the study population was 13.3 seconds (SD 5.7). This value was similar to previous studies in older people with mobility problems[315]. The mean time taken to perform the TGUG was 14.4 seconds (SD 4.6) in patients with a falls history. The time taken to do the TGUG was 0.2 seconds less in the spironolactone group compared to placebo group at 10 weeks (p=0.72) with an insignificant change of 0.04 seconds at 20 weeks (p=0.95). At baseline the placebo group took 0.4 seconds longer to perform the TGUG test (p=0.69). After adjusting for baseline TGUG and age as covariates there were still no significant changes in the time taken to perform the test at both 10 weeks (p=0.8) and 20 weeks (p=0.87) as shown in Table 3.9.

Table 3.9 – Mean change in the Timed-Get-Up and Go test.

Outcome measure	Time	Spironolactone (95% CI)	Placebo (95%CI)	Difference between groups [Spironolactone - Placebo] (95% CI)	P value
Change in TGUG (s) Unadjusted	10 weeks	-1.4 (-2.2 to -0.6)	-1.2 (-2.0 to -0.4)	-0.2 (-1.3 to 0.9)	0.72
	20 weeks	-1.3 (-2.2 to -0.3)	-1.3 (-2.3 to -0.4)	0.04 (-1.3 to 1.3)	0.95
Change in TGUG (s) Adjusted*	10 weeks	-1.4 (-2.1 to -0.7)	-1.3 (-1.9 to -0.6)	-0.1 (-1.1 to0.8)	0.80
	20 weeks	-1.3 (-1.9 to -0.6)	-1.4 (-2.2 to -0.5	0.1 (-1.1 to 1.3)	0.87

* Adjusted model using baseline TGUG and age as covariates

Health Related Quality of Life and psychological state

Table 3.10 shows the change in EuroQol EQ-5D and EQ-VAS from baseline. A rise in EQ-5D score or EQ-VAS indicates an improvement in quality of life. There was a highly significant difference in the EQ-5D utility score of 0.1 (95% CI 0.03 to 0.18) in the spironolactone compared to the placebo group at 20 weeks (p=0.006). Although not statistically significant, the baseline EQ-5D utility score was 0.08 lower in the spironolactone group compared to the placebo group. Despite adjusting for baseline EQ-5D and age as covariates there was still a significant difference between the groups, with a mean change of 0.07 (95% CI 0.01 to 0.13) in the spironolactone group compared to the placebo group at 20 weeks (p=0.04). Within the EuroQol EQ-5D subgroups the most significant change was found within the pain/discomfort domain ($p<0.01$), with joint pain due to osteoarthritis as the main cause of pain. Change in the EQ-5D in the spironolactone group did not correlate to a change in 6MWT distance at 20 weeks (Pearson's r=0.16, p=0.09).

Although there was a slight improvement in the spironolactone group at 10 weeks, in the EQ-VAS questionnaire this change was not significant. There was no significant difference in the change of EQ-VAS between the two groups at 10 weeks or 20 weeks.

The Hospital Anxiety and Depression Score (HADS) is a useful measure of psychological status in patients. According to Zigmond et al the cut-off scores for detecting anxiety and depression a HADS score of 8-10 suggest borderline cases and scores >11 suggest a valid diagnosis of anxiety or depression[356]. In this study 6% (7/120) of participants reached the threshold for a diagnosis of anxiety but only 2% (2/120) reached the threshold for a diagnosis of depression with 12% of participants

having borderline depression. Table 3.11 shows the change in HADS score from baseline. There was a significant fall in the HADS-D score of 0.84 in the spironolactone group compared to placebo group at 10 weeks (p=0.03) i.e. an improvement in mood. However, no significant between group difference was found in HADS-D score at 10 weeks after performing analysis adjusting for baseline score and age as covariates (p=0.11). There was no significant difference between groups in HADS-D score at 20 weeks. No significant between group difference was seen in HADS-A score at 10 weeks or 20 weeks.

In the Functional Limitation Profile (FLP) questionnaire the mean baseline FLP score was high in both groups, with a total FLP score of 807 (SD 198) in the spironolactone group compared to 773 (SD 180) in the placebo group. A rise in FLP score indicates increased functional impairment. There were no significant differences in the change in total FLP scores between groups at either 10 weeks and 20 weeks (Table 3.12). There were also no significant differences found between groups in the physical and psychosocial domains of the questionnaire during the study. After adjusting for baseline total FLP score and age as covariates, the change in total FLP score between groups was 13 (95%CI -40 to 66) at 20 weeks in the spironolactone compared to placebo, but this was not statistically significant (p=0.62).

Table 3.10 – Mean change in the EQ-5D and EQ-VAS from baseline, unadjusted and adjusted measures.

Outcome measure	Time	Spironolactone (95% CI)	Placebo (95%CI)	Difference between groups [Spironolactone - Placebo] (95% CI)	P value
Change in EQ-5D Unadjusted	10 weeks	0.05 (0.04 to 0.10)	0.01 (-0.04 to 0.06)	0.04 (0.03 to 0.11)	0.22
	20 weeks	0.11 (0.06 to 0.16)	0.01 (-0.05 to 0.06)	0.10 (0.03 to 0.18)	**0.006**
Change in EQ-5D Adjusted*	10 weeks	0.04 (-0.01 to 0.08)	0.03 (-0.02 to 0.07)	0.01 (-0.06 to 0.07)	0.81
	20 weeks	0.09 (0.05 to 0.14)	0.02 (-0.02 to 0.07)	0.07 (0.01 to 0.13)	**0.04**
Change in EQ-VAS Unadjusted	10 weeks	4.2 (0.2 to 8.3)	2.8 (-1.3 to 6.8)	1.5 (-4.3 to 7.2)	0.62
	20 weeks	-0.3 (-4.8 to 4.2)	1.6 (-3.0 to 6.2)	-1.9 (-8.3 to 4.5)	0.55
Change in EQ-VAS Adjusted †	10 weeks	4.3 (0.6 to 7.9)	2.7 (-0.9 to 6.4)	1.6 (-3.6 to 6.7)	0.61
	20 weeks	-0.4 (-4.4 to 3.7)	1.6 (-2.5 to 5.7)	-2.0 (-7.8 to 3.8)	0.57

A rise in EQ-5D or EQ-VAS score indicates improvement in quality of life.

*Adjusted model using baseline EQ-5D and age as covariates

† Adjusted model using baseline EQ-VAS and age as covariates

Table 3.11 – Mean change in HADS-A and HADS-D score from baseline, unadjusted and adjusted measures.

Outcome measure	Time	Spironolactone (95% CI)	Placebo (95%CI)	Difference between groups [Spironolactone - Placebo] (95% CI)	P value
Change in HADS-A score Unadjusted	10 weeks	-0.11 (-0.79 to 0.57)	-0.23 (-0.91 to 0.45)	0.12 (-0.83 to 1.09)	0.80
	20 weeks	0.33 (-0.35 to 0.96)	-0.45 (-1.12 to 0.21)	0.78 (-0.17 to 1.69)	0.11
Change in HADS-A score Adjusted*	10 weeks	-0.14 (-0.80 to 0.53)	-0.20 (-0.87 to 0.46)	0.07 (-0.87 to 1.01)	0.89
	20 weeks	0.28 (-0.35 to 0.90)	-0.43 (-1.06 to 0.21)	0.70 (-0.19 to 1.60)	0.12
Change in HADS-D score Unadjusted	10 weeks	-1.11 (-1.61 to -0.56)	-0.25 (-0.77 to 0.28)	-0.84 (-1.58 to -0.09)	**0.03**
	20 weeks	-0.23 (-0.78 to 0.31)	-0.04 (-0.59 to 0.51)	-0.20 (-0.97 to 0.58)	0.62
Change in HADS-D score Adjusted*	10 weeks	-0.96 (-1.44 to -0.47)	-0.38 (-0.87 to 0.11)	-0.57 (-1.27 to 0.12)	0.12
	20 weeks	-0.14 (-0.68 to 0.39)	-0.13 (-0.66 to 0.41)	-0.02 (-0.78 to 0.74)	0.95

A rise in HADS-A and HAD-D indicates worsening symptoms of anxiety or depression.

* Adjusted model using baseline measures and age as covariates.

Table 3.12– Mean change in Functional Limitation Profile (FLP) domains from baseline, unadjusted measures.

	Time	Spironolactone (95% CI)	Placebo (95%CI)	Difference between groups [Spironolactone - Placebo] (95% CI)	P value
Change in FLP Total Score	10 weeks	-16 (-49 to 17)	-17 (-52 to 14)	1 (-45 to 47)	0.97
	20 weeks	-14 (-54 to 25)	-22 (-52 to 14)	8 (-49 to 64)	0.78
Change in FLP Physical domain	10 weeks	-10 (-24 to 4)	-8 (-22 to 5)	-2 (-21 to 18)	0.86
	20 weeks	-1 (-16 to 13)	-5 (-20 to 9)	4 (-17 to 25)	0.71
Change in FLP Psychosocial Domain	10 weeks	-15 (-34 to 5)	-6 (-26 to 13)	-8 (-36 to 20)	0.56
	20 weeks	-1 (-23 to 21)	-9 (-32 to 13)	-8 (-23 to 40)	0.58

An increased FLP score indicates increased severity of functional impairment.

3.4 Changes in Blood Pressure

There was a significant fall in systolic and diastolic blood pressure with spironolactone at 10 weeks, as shown in Table 3.13. The systolic blood pressure in the spironolactone group fell by 7.3 (95% CI 1.3 to 13.4) mmHg relative to the placebo group at 10 weeks (p=0.02). Despite adjusting for baseline systolic blood pressure as a covariate, systolic blood pressure was still lower in the spironolactone compared to placebo group at 10 weeks (p=0.03). There was a significant fall in diastolic blood pressure in the spironolactone group at 10 weeks, with a mean difference of -5.3 (95% CI -9 to -1.5) mmHg in the spironolactone group compared to placebo group. However, there was no significant difference between groups after adjusting for baseline diastolic blood pressure (p=0.053). Non-significant falls were seen in both systolic and diastolic blood pressure in the spironolactone group at 20 weeks (Figures 3.4 and 3.5).

There was no significant fall in postural blood pressure with spironolactone compared to placebo at 20. The mean postural fall in systolic blood was 5 mmHg (SD 15) in the spironolactone group compared to 2 mmHg (SD 16) in the placebo group at 20 weeks (p=0.36). The mean postural fall in diastolic blood pressure in the spironolactone and placebo group was minimal at 20 weeks (-0.8mmHg vs. 0.8mmHg respectively) (p=0.26). Systolic blood pressure fell by >20 mmHg on standing in 7 participants in the spironolactone group and 2 participants in the placebo group (p=0.59). Diastolic blood pressure fell by >10 mmHg on standing in 7 patients in the spironolactone group and 6 patients in the placebo group (p=0.96).

Table 3.13– Mean change in systolic and diastolic blood pressure from baseline, both adjusted and unadjusted for baseline measures.

Outcome measure	Time	Spironolactone (95% CI)	Placebo (95% CI)	Difference between groups [Spironolactone - Placebo] (95% CI)	P value
Change in Systolic Blood pressure (mmHg) Unadjusted	10 weeks	-7.7 (-11.9 to -3.5)	-0.32 (-4.6 to 4.0)	-7.3 (-13.4 to -1.3)	**0.02**
	20 weeks	-9.3 (-14.0 to -4.5)	-2.6 (-7.4 to 2.2)	-6.6 (-13.4 to 0.1)	0.055
Change in Systolic blood pressure (mmHg) Adjusted for baseline*	10 weeks	-7.1 (-10.9 to -3.1)	-0.9 (-4.9 to 3.0)	-6.1 (-11.6 to -0.6)	**0.03**
	20 weeks	-8.5 (-12.7 to 4.3)	-3.4 (-7.6 to 0.9)	-5.1 (-11.1 to 0.8)	0.09
Change in Diastolic blood pressure (mmHg) Unadjusted	10 weeks	-4.6 (-7.2 to -2.0)	0.7 (-2.0 to 3.4)	-5.3 (-9.0 to -1.5)	**<0.01**
	20 weeks	-4.8 (-7.5 to -2.2)	-1.7 (-4.4 to 1.0)	-3.1 (-6.9 to -0.6)	0.10
Change in Diastolic blood pressure (mmHg) Adjusted for baseline†	10 weeks	-3.5 (-5.7 to -1.3)	-0.4 (-2.6 to 1.8)	-3.1 (-6.3 to 0.1)	0.053
	20 weeks	-3.8 (-6.0 to -1.6)	-2.8 (-5.0 to -0.5)	-1.1 (-4.2 to 2.1)	0.50

*Analysis performed using baseline systolic blood pressure as a covariate

†Analysis performed using baseline diastolic blood pressure as a covariate

Figure 3.4 – Mean change in systolic blood pressure at 10 and 20 weeks in spironolactone and placebo groups. Vertical lines show 95% CI.

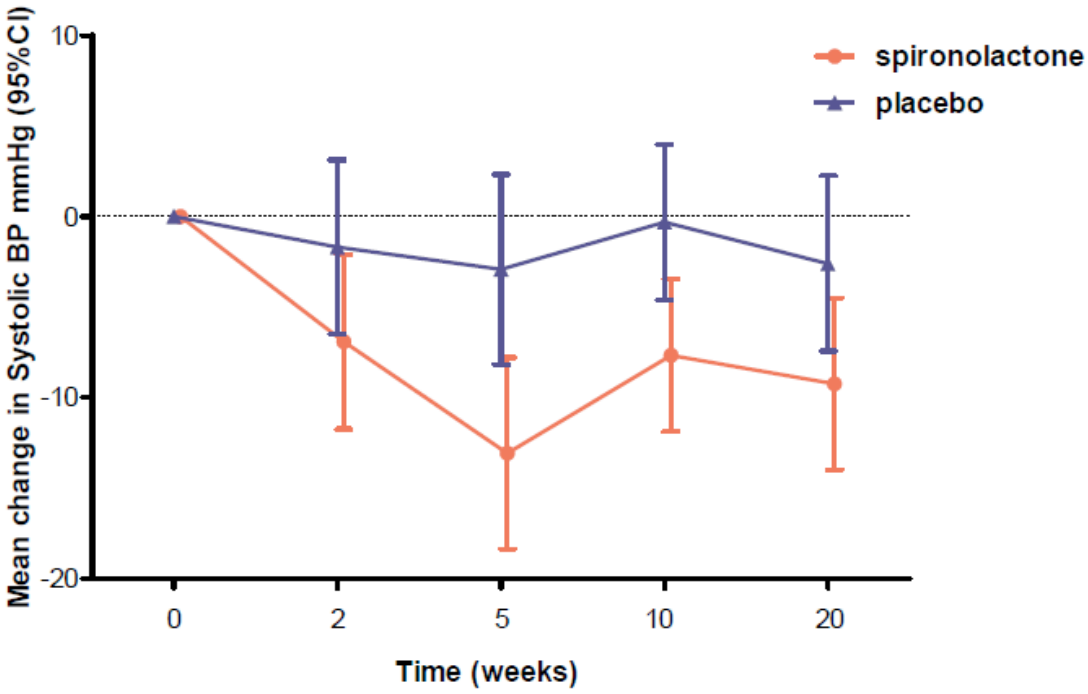
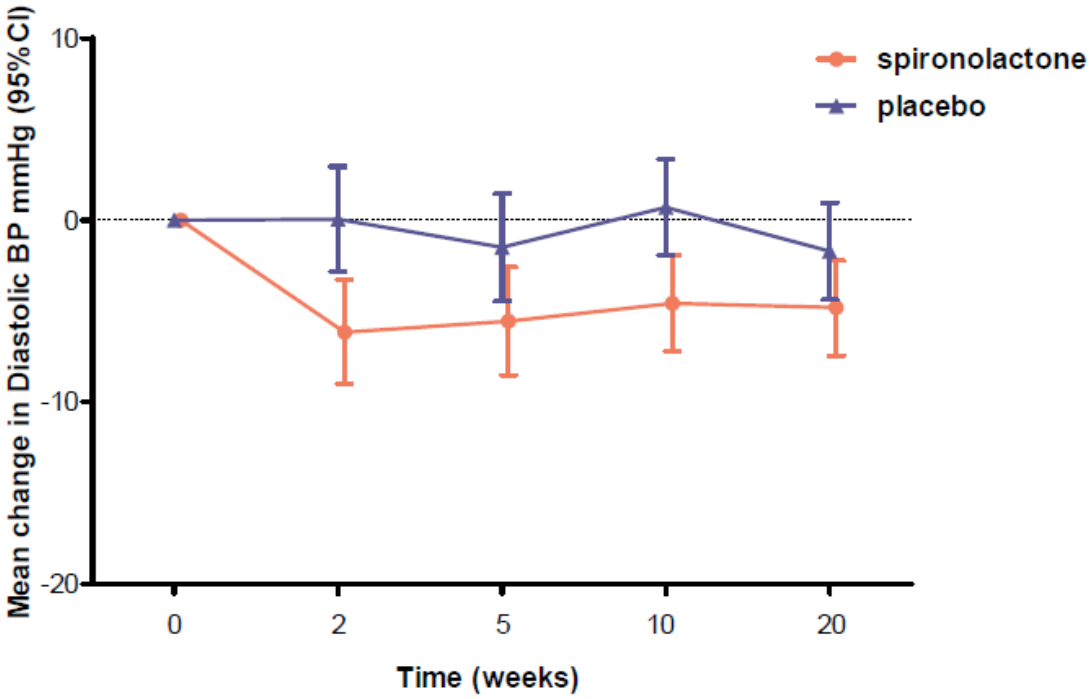


Figure 3.5 – Mean change in diastolic blood pressure at 10 and 20 weeks in spironolactone and placebo groups. Vertical lines show 95% CI.



3.5 Changes in renal function and serum magnesium

Table 3.14 summarises the changes in blood tests from the baseline in the spironolactone and placebo groups. Spironolactone was associated with a significant rise in serum potassium with a between group difference of 0.2 (95% CI 0.1 to 0.3) mmol/L compared to placebo group at 20 weeks ($p<0.01$). Figure 3.6 shows the mean serum potassium in both groups over the 20 weeks.

Spironolactone was also associated with a small but significant increase in serum creatinine at both 10 weeks and at 20 weeks. Analysis showed a 9% increase in mean serum creatinine from baseline in the spironolactone group compared to a 3% increase in the placebo group at 20 weeks ($p=0.03$). Mean baseline serum creatinine was higher in those aged >80 years compared to those <80 years old (80 mmol/L vs. 74 mmol/L), ($p=0.30$). Despite adjusting analyses using age and sex as covariates, there was still a significant increase in serum creatinine in with spironolactone with a between group difference of 4.2 (95% CI 0.4 to 7.9) mmol/L in the spironolactone group compared to placebo group at 20 weeks ($p=0.03$). There was no significant difference found in serum urea between the two groups at both 10 weeks and 20 weeks.

Although there was an initial fall in serum sodium at 10 weeks with the mean difference in the spironolactone group of -1.3 mmol/L (95% CI -2.3 to -0.3) relative to the placebo group ($p=0.02$), this was not sustained by 20 weeks. There was no significant change in serum magnesium between groups at both 10 weeks and 20 weeks.

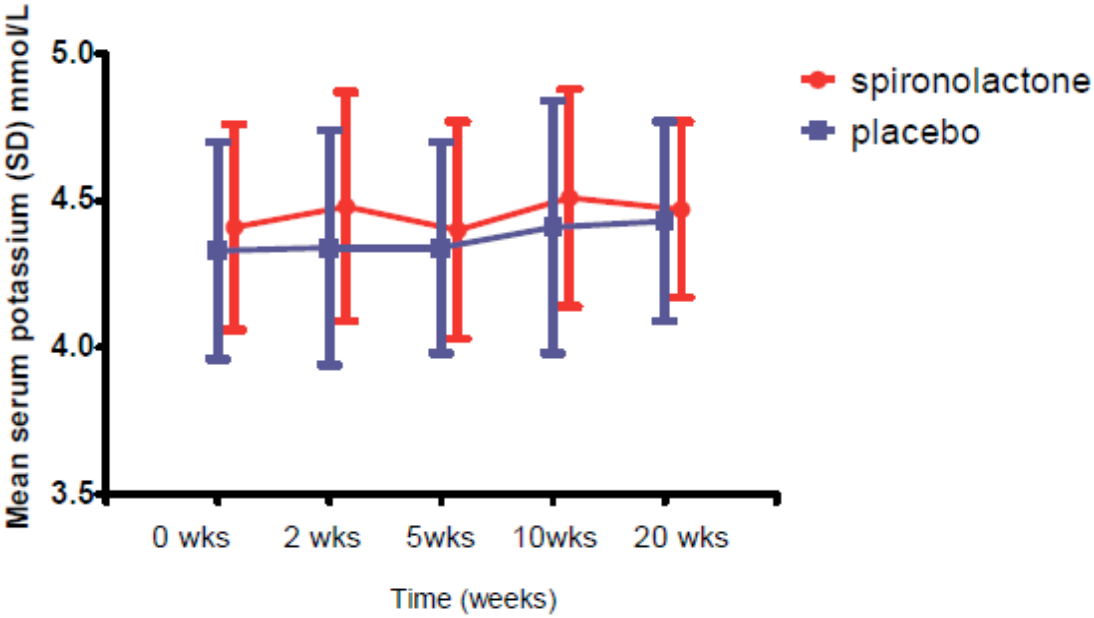
The changes in serum sodium, potassium and creatinine are similar to changes found in participants taking spironolactone in the RALES study[262]. However, unlike the RALES

study, none of the participants on spironolactone in this study had medication discontinued due to serious hyperkalaemia (K^+ >5.5mmol/L). This was probably due to the fact that participants in the RALES study were already taking an ACE inhibitor and had heart failure. Spironolactone was well tolerated in this study involving older people.

Table 3.14. The changes in renal function and serum magnesium from baseline.

Assay	Time	Spironolactone (95%CI)	Placebo (95%CI)	P value
Serum potassium (mmol/L)	Change at 10 weeks	0.2 (0.1 to 0.3)	0.0 (-0.1 to 0.0)	**<0.01**
	Change at 20 weeks	0.2 (0.1 to 0.3)	0.0 (-0.1 to 0.1)	**<0.01**
Serum Sodium (mmol/L)	Change at 10 weeks	-1.4 (-2.2 to -0.7)	-0.2 (-0.9 to 0.5)	**0.02**
	Change at 20 weeks	-0.9 (-1.6 to -0.3)	-0.6 (-1.2 to 0.1)	0.43
Serum Creatinine (umol/L)	Change at 10 weeks	6.3 (4.1 to 8.6)	1.0 (-1.2 to 3.2)	**<0.01**
	Change at 20 weeks	6.7 (6.1 to 9.4)	2.5 (-0.2 to 5.2)	**0.03**
Serum urea (mmol/L)	Change at 10 weeks	0.5 (0.1 to 0.8)	0.2 (-0.2 to 0.5)	0.18
	Change at 20 weeks	0.5 (0.2 to 0.9)	0.4 (0.0 to 0.7)	0.67
Serum Magnesium (mmol/L)	Change at 10 weeks	-0.02 (-0.03 to 0.00)	-0.02 (-0.03 to 0.00)	0.57
	Change at 20 weeks	-0.02 (-0.04 to -0.01)	-0.03 (-0.05 to -0.01)	0.55

Figure 3.6 – Mean serum potassium in the spironolactone and placebo groups. Vertical lines represent SDs.



3.6 Changes in B-type Natriuretic Peptide and Aldosterone

Data at baseline and 20 weeks for both BNP and aldosterone were not normally distributed and the between groups comparisons were performed using Mann Whitney tests. As data for change in BNP and aldosterone at 20 weeks were normally distributed, analyses for changes between groups were performed using Student's t-tests.

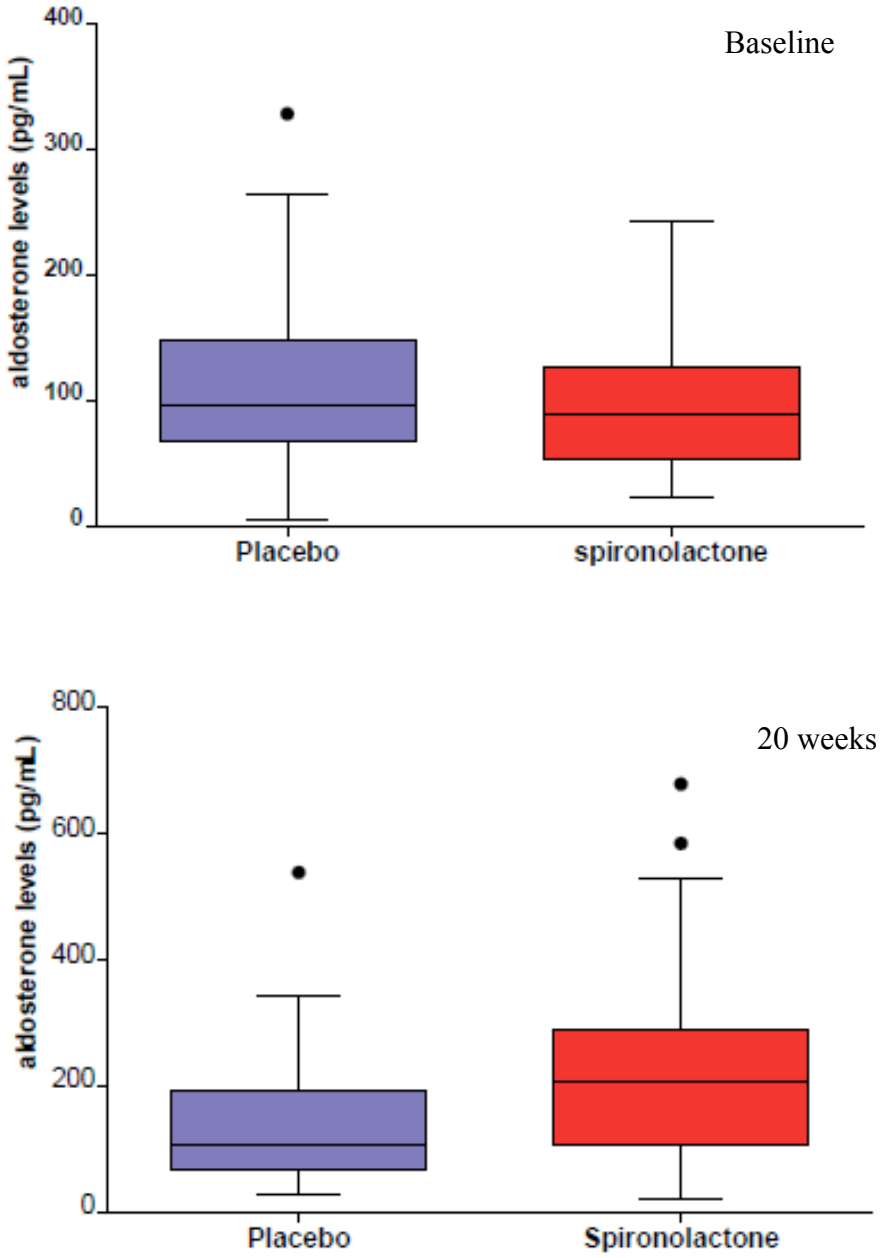
Spironolactone was associated with a significant rise in aldosterone, with an increase of 116.8 pg/ml (95%CI 166.1 to 167.6) compared to placebo at 20 weeks ($p<0.01$). Despite adjusting for baseline aldosterone as a covariate, spironolactone was associated with a 60% increase in serum aldosterone from baseline, with a between group difference of 112.0 pg/ml (95%CI 60.0 to 164.0) compared to placebo group at 20 weeks ($p<0.01$). The rise in plasma aldosterone with spironolactone correlated with the rise in serum potassium (Pearson's $r=0.30$, $p=0.002$), in keeping with aldosterone blockade. No correlation was found between changes in aldosterone levels and change in 6MWT distance ($r=0.11$, $p=0.25$) at 20 weeks. Figure 3.7 shows the median aldosterone levels at baseline and 20 weeks in both groups.

Although BNP levels did fall by 3.0 pg/ml (95% CI -7.5 to 13.6) in the spironolactone group compared to placebo at 20 weeks, this was not significant. Baseline BNP did not correlate with any other outcome or baseline characteristic.

Table 3.15– Changes in plasma BNP and aldosterone levels at 20 weeks

Assay	**Time**	**Spironolactone (95% CI) n=57**	**Placebo (95% CI) n=55**	**Difference between groups [Spironolactone -Placebo] (95% CI)**	**P value**
BNP (pg/ml)	Change at 20 wks	-4.2 (-11.6 to 3.3)	-1.2 (-8.6 to 6.4)	3.0 (-7.5 to 13.6)	0.57
Aldosterone (pg/ml)	Change at 20 wks	136.9 (101.3 to 172.7)	20.1 (-15.9 to 56.2)	116.8 (66.1 to 167.6)	**0.001**

Figure 3.7 – Plasma aldosterone levels in spironolactone and placebo groups at baseline and 20 weeks. Boxes are represent median and IQRs. Vertical lines represent range of results.



Outliers are denoted by ●

3.7 Changes in plasma cortisol levels

Plasma cortisol levels were measured at baseline and 20 weeks to investigate the effects of mineralocorticoid receptor blockade on glucocorticoid levels. Cortisol levels are known to fluctuate throughout the day, with peak levels early morning around 8am and trough levels around midnight. Due to the diurnal variation of plasma cortisol levels, the statistical analysis of cortisol was stratified according to the time of day the blood samples were taken at baseline and 20 weeks in each individual. Blood samples were taken at two different times of day; mid-morning (around 11am) or mid-afternoon (around 3pm). Of the 112 participants who completed the study, 17 participant samples were excluded from the analysis. Reasons for exclusion were as follows: blood samples were taken at a different time of day at baseline and 20 weeks (n=15) and volume of sample insufficient to perform the analysis (n=2). Thus, cortisol levels were analysed at baseline and 20 weeks in the remaining 95 participants. Although the mean plasma cortisol levels were higher in the spironolactone group compared placebo at baseline, the difference in plasma cortisol levels between groups at baseline was not statistically significant (p=0.32). Thirty-five participants had their cortisol levels checked mid-morning at baseline and 20 weeks, with a between group difference higher in the placebo group compared to the spironolactone group. Due to the small rise in cortisol levels in the spironolactone group mid-morning there was no significant between group change in cortisol levels at 20 weeks (p=0.26) (Table 3.16).

In contrast, to the mid-morning samples, spironolactone caused a significant rise in plasma cortisol mid-afternoon, with a between group difference of 78.4 nmol/L (95% CI 22.8 to 133.9) (p=0.007). Despite adjusting analysis for baseline, cortisol levels there was still a significant rise in plasma cortisol of 77.2 nmol/L (95% CI 24.0 to 130.5) in

the spironolactone group compared to the placebo group (p=0.005). The rise in plasma cortisol was similar to the rise in cortisol seen in hypertensive diabetic patients after 4 weeks of spironolactone[250]. No significant correlation was shown between change in mid-afternoon cortisol levels and change in 6MWT distance (Pearson's r= -0.11, p=0.37).

Table 3.16 – Change in plasma cortisol between groups at 20 weeks

Assay	**Time**	**Spironolactone (95% CI)**	**Placebo (95% CI)**	**Difference between groups [Spironolactone – Placebo] at 20 weeks (95% CI)**	**P value**
Cortisol (nmol/L) 11 am (n=35)	Baseline	285.0 (238.6 to 333.8)	250.0 (203.4 to 304.2)	-51.3 (-142.4 to 39.9)	0.26
	Change at 20 weeks	19.1 (-48.1 to 86.3)	70.3 (8.6 to 131.2)		
Cortisol (nmol/L) 3pm (n=60)	Baseline	266.2 (226.9 to 295.1)	269.5 (232.8 to 304.8)	78.4 (22.8 to133.9)	**0.007**
	Change at 20 weeks	66.1 (27.1 to105.1)	-12.3 (-51.9 to 27.3)		

3.8 Adverse events

A total of 16 adverse events occurred to 8 in the placebo group and 8 in the spironolactone group. Table 3.17 summarises the reasons for participants developing adverse events during the study. Of the participants who completed the trial 95% (106/112) remained on medication at 20 weeks.

Six participants had their study medication discontinued due to adverse events. Medication was discontinued in 4 participants in the spironolactone group: serum sodium <130mmol/L (n=2), fall in systolic blood pressure <100mmHg (n=1) and skin rash (n=1). Medication was discontinued in 2 participants in the placebo group: persistent dizziness (n=1), skin rash (n=1). Two thirds of the participants whose medication was discontinued remained in the study and completed the outcomes at 20 weeks in order to preserve intention to treat analysis.

Five participants (2 in the spironolactone group and 3 in the placebo group) were unable to tolerate up-titration of dose at 2 weeks due to a rise in serum potassium (>5.0 mmol/L but <5.5 mmol/L). All 5 participants remained on the lower medication dose (12.5mg spironolactone/placebo) for the duration of the study. No individual experienced serious hyperkaelaemia (potassium >5.5 mmol/L).

Two participants in the placebo group experienced serious adverse events during the study. One was hospitalised for septic arthritis prior to starting the study medication and the second was admitted to hospital for an elective hip replacement for severe osteoarthritis and did not wish to continue the medication after the operation.

Table 3.17 Reasons for adverse events

Adverse event	Spironolactone (n=8)	Placebo (n=8)
Serious adverse events Hospitalisation	0	2 Hip Arthroplasty (1) Septic Arthritis (1)
Adverse events Rash	1	1
Abdominal discomfort	1	0
Hyponatraemia	2	0
Dizziness	0	1
Fall	0	1
Hypotension	1	0
Gynaecomastia	1	0
Rise in potassium >5.0mmol/L but <5.5mmol/L	2*	3*
Serious hyperkalaemia Potassium >5.5mmol/L	0	0

* Patients remained in the study on a lower dose of spironolactone/placebo.

3.9 Adherence to study medication

Adherence to study medication was assessed by tablet counting with "Good adherence" >85% of the expected value. The median adherence was 99% in both groups with 89% of the spironolactone group and 94% of the placebo group achieving >85% adherence to medication, as shown in Table 3.18. Participants with a tablet count lower than expected were assumed to have taken extra tablets in error. Thirty percent of participants in the spironolactone group took extra tablets with no apparent serious adverse effects.

Table 3.18 – Adherence to medication in both groups at 20 weeks.

	Spironolactone (n= 54)	**Placebo (n= 53)**
Median Adherence % (Range)	99 (44-121)	99 (51-110)
No. of participants with >85% adherence of expected (%)	48 (89)	50 (94)
No of participants who missed > 25% of tablets (%)	3 (6)	2 (4)
No. of patients taking extra tablets (%)	16 (30)	9 (17)

3.10 Subgroup Analysis

Primary Outcome

Initial analyses of the primary outcome showed there was no significant difference in change in 6MWT distance with spironolactone compared to placebo. However, it is possible that any potential effect of spironolactone on change in 6MWT distance could have been masked by factors such as variation of age or sex within the study population. Several other factors may have also blunted the effects of spironolactone. Firstly, the use of other medications which alter potassium balance such as loop and thiazide diuretics may affect muscle contractility. Secondly, as spironolactone is widely used in patients with heart failure and hypertension, the effects within these participants may differ from those without hypertension. Thirdly, the effects of spironolactone at lowering blood pressure may have been potentiated when used in combination with other antihypertensive medications or when given to participants with disease processes affecting the autonomic nervous system (e.g. Parkinson's disease). Fourthly, the effects of spironolactone may have been offset by the use of synthetic steroids.

There was also a small but non-significant difference between the two groups at baseline in severity of mobility problems as measured by the number of people who used walking aids. Fewer participants in the placebo group used a walking aid which may have been a factor in the slight improvement in 6MWT distance in the placebo group at 20 weeks. The subgroups were analysed using fixed effect ANCOVA test to evaluate if there was any factor which contributed to the lack of effect of spironolactone on change in walking distance. Table 3.19 shows the subgroup analyses. As numbers in the subgroup analyses were small it is difficult to achieve statistical significance. From

the fixed effect comparison between groups there was a deterioration in the change in walking distance in spironolactone group relative to the placebo group, depending on whether participants had a systolic blood pressure <140 mmHg at baseline or if they had no previous history of hypertension. However, these differences were not statistically significant. There was also an indication that participants in the spironolactone group showed a decline in change of walking distance relative to the placebo group if participants were taking oral diuretics or corticosteroids. However, due to the small numbers within the subgroups neither these results were not statistically significant. In order to test these subgroup hypotheses further a much larger study population would be required.

Table 3.19 – Subgroup analysis of factors that could affect the change in baseline 6MWT distance (between group difference between spironolactone and placebo)

Subgroup	No. of subjects (n=)	Mean difference in 6MWT distance between groups	95% CI	P value	Comparison between subgroups P value
All patients	112	-3.2	(-38.9 to 22.5)	0.81	
Male	61	-11.3	(-42.3 to 19.7)	0.47	0.44
Female	51	5.7	(-38.1 to 49.5)	0.79	
Walking aids	35	-15.2	(-43.6 to 73.9)	0.60	0.89
No walking aids used	77	-11.2	(-39.1 to 16.7)	0.43	
Age <80 years	84	-1.1	(-45.1 to 43.0)	0.96	0.83
Age >80 years	28	-4.2	(-35.8 to 27.4)	0.79	
Oral diuretics	24	-16.6	(-55.5 to22.3)	0.38	0.23
Loop diuretics	6	-30.3	(-138.8 to77.5)	0.48	
Thiazide diuretics	18	2.3	(-55.1 to 59.6)	0.93	
Not on diuretics	88	2.9	(-27.0 to32.8)	0.85	
Taking antihypertensive medication (including diuretics)	33	0.3	(-39.3to39.9)	0.98	0.73
Not taking antihypertensive medication (including diuretics)	79	-13.0	(-40.4to39.9)	0.34	
On B-blockers	7	-13.3	(-120.9 to44.3)	0.75	0.85
Not on B-blockers	105	-2.2	(-28.8 to 24.3)	0.87	
On Ca^{2+} channel blockers	15	0.79	(-56.1 to 57.7)	0.47	0.74
Not on Ca^{2+} channel blockers	97	-11.1	(-35.5 to13.3)	0.37	
On alpha blockers	8	-0.4	(-114.2 to13.4)	0.99	0.96
Not on alpha blockers	104	-3.9	(-30.2 to 23.1)	0.78	

SBP >140 mmHg	73	-6.1	(-29.3 to 17.1)	0.60	0.43
SBP <140mmHg	37	-33.9	(-77.1 to 9.3)	0.12	
History of hypertension	32	20.0	(-40.2 to 80.3)	0.5	0.49
No history of hypertension	80	-13.3	(-41.0 to14.4)	0.34	
History of osteoarthritis	38	-28.1	(-64.1 to7.8)	0.12	0.35
No previous history of osteoarthritis	74	7.1	(-20.9 to 35.3)	0.61	
No history of Parkinson's Disease	105	11.7	(-124.6 to 147.9)	0.84	0.87
History of Parkinson's Disease	7	-3.6	(-30.7 to 23.5)	0.79	
Rise in serum K^+ during the study	62	-6.1	(-37.1 to14.5)	0.39	0.43
No rise in serum K^+ during the study	50	-5.3	(-51.2 to40.4)	0.81	
Rise is plasma aldosterone during the study	81	-14.7	(-49.6 to20.3)	0.40	0.51
No rise in plasma aldosterone during the study	31	10.4	(-36.5 to57.3)	0.65	
A fall in BNP during the study	59	-9.0	(-34.9 to 16.8)	0.65	0.75
A rise in BNP during the study	53	-1.2	(-54.0 to 51.5)	0.96	
Baseline creatinine clearance <60ml/min	28	-12.5	(-37.9 to 12.9)	0.33	0.58
Baseline creatinine clearance>60ml/min	84	-1.6	(-50.0 to 46.7)	0.93	
Not taking corticosteroids	104	-4.9	(-27.5 to17.7)	0.67	0.35
Taking corticosteroids	8	-41.3	(-128.9 to 46.3)	0.1	
Participants recruited from MFE services	14	19.9	(-44.2 to 83.9)	0.51	0.55
Participants recruited from Primary Care	106	-5.4	(-33.5 to 22.6)	0.7	

EuroQol (EQ-5D)

Initial analysis showed there was a significant improvement in quality of life as measured by improvement in EQ-5D utility scores in the spironolactone group compared to placebo group at twenty weeks, after adjusting for baseline EQ-5D scores and age as covariates ($p=0.04$).

The EQ-5D utility score comprises of 5 domains including mobility, self-care, usual activities, pain / discomfort and anxiety / depression. The most significant change within the domains of the EQ-5D was found in pain / discomfort, with 35% (20/57) of participants in the spironolactone group showing improvement in pain compared to 5% (3/55) of those in the placebo group at 20 weeks ($p<0.01$) (Table 3.20)

Subgroup analyses were done to examine which particular subgroup improved quality of life (EQ-5D) relative to placebo (Table 3.21). It is possible that the beneficial effects of spironolactone in improving quality of life could have been age or gender dependent. Secondly, as one of the most important factors in the improvement of EQ-5D was due to improvement in pain perception, it is possible that spironolactone may have a beneficial role in inflammatory diseases such as osteoarthritis and rheumatoid arthritis. Thirdly, as quality of life includes not only physical but psychological health status, it is possible that participants with established mood disorders may show variations in quality of life scores in contrast to those without mood disorders.

A comparison of various subgroups was done using fixed effect ANCOVA to evaluate if any hypothesis for the improvement in EQ-5D utility score in the spironolactone group could be generated.

From the fixed effect comparison between subgroups suggests that spironolactone might possibly be more effective at improving quality of life in participants aged <80 years old who require a walking aid. There was also a statistically significant improvement in quality of life in participants with osteoarthritis in the spironolactone group compared to placebo group (p=0.02). However, the fixed effect comparison was not significant when comparing this to participants without osteoarthritis, possibly due to small subgroup numbers. Those in the spironolactone group were also found to have improved quality of life if they were already taking analgesia and, in particular, if they were taking opioid analgesia (p=0.03). This may also support the hypothesis of the potential anti inflammatory effects of spironolactone. However, as this study was not powered to detect this effect, no firm conclusions can be made. Finally, in contrast to our initial hypothesis spironolactone did not improve quality of life in participants taking antidepressant medication. This may be due to the relatively small numbers of participants with a diagnosis of depression in this study. In conclusion, a larger study would be required to test this hypothesis further.

Table 3.20 – Change in EQ-5D domain scores at 20 weeks between groups.

EQ-5D domains	**Spironolactone No. of participants with improved EQ-5D subgroup scores at 20 weeks (%) (n=57)**	**Placebo No. of participants with improved EQ-5D subgroup scores at 20 weeks (%) (n=55)**	**P value**
Mobility domain	19/57 (33)	12/55 (22)	0.15
Self-care domain	10/57 (18)	7/55 (13)	0.47
Usual activities domain	14/57 (25)	17/55 (31)	0.77
Pain/Discomfort domain	20/57 (35)	3/55 (5)	**<0.01**
Anxiety/Depression domain	5/57 (8)	6/55 (10)	0.61

Changes in EQ-5D subgroups were discrete values therefore between group significance was analysed by Pearsons Chi squared test.

Table 3.21 – Subgroup analysis for factors that could affect change in EQ-5D

Subgroup	No. of subjects (n=)	Mean difference in EQ-5D (Spironolactone-Placebo)	95% CI	P value	Comparison between subgroups P value
Male	61	0.10	(0.01 to 0.2)	0.06	0.96
Female	51	0.11	(0.01 to 0.2)	0.06	
Walking aids	35	0.22	(0.05 to 0.40)	**0.01**	**0.05**
No walking aids	77	0.07	(0.1 to 0.14)	0.08	
Age <80 years	84	0.10	(0.01 to 0.19)	**0.03**	0.94
Age >80 years	28	-0.10	(-0.21 to 0.03)	0.06	
Taking antidepressants	9	-0.10	(-0.47 to 0.27)	0.54	0.09
Not taking antidepressants	103	0.12	(0.05 to 0.20)	0.01	
History of hypertension	33	0.13	(-0.03 to 0.29)	0.11	0.59
No history of hypertension	79	0.09	(0.01 to 0.17)	0.04	
History of IHD	32	0.17	(-0.13 to 0.47)	0.25	0.49
No history of IHD	80	0.09	(0.02 to 0.17)	0.02	
History of PD	7	0.09	(-0.65 to 0.83)	0.77	0.94
No history of PD	105	0.11	(0.03 to 0.18)	0.01	
History of osteoarthritis	38	0.16	(0.03 to 0.30)	**0.02**	0.33
No history of arthritis	74	0.08	(-0.01 to 0.18)	0.08	
Taking analgesia at baseline	43	0.18	(0.02 to 0.35)	**0.03**	0.12
Paracetamol	8	0.04	(-0.34 to 0.41)	0.82	
NSAIDs	8	0.13	(-0.29 to 0.54)	0.49	
Opioids	27	0.30	(0.11 to 0.48)	**0.003**	
Not taking analgesia at baseline	62	0.06	(-0.01 to 0.14)	0.09	
Rise in plasma aldosterone levels during the study	81	0.12	(0.02 to 0.22)	**0.02**	0.25
No rise in plasma aldosterone levels during the study	31	-0.01	(-0.1 to 0.08)	0.80	

4. DISCUSSION

This study found that spironolactone did not improve physical function compared to placebo, as measured by the change in 6MWT distance at 20 weeks. The observed difference was 3.2 metres less in the spironolactone group compared to the placebo group at 20 weeks. This was much lower than the 30 metre distance required to produce a clinically significant effect. As shown by the 95% confidence intervals, which do not include a 30 metre improvement, it is highly unlikely that a clinically significant improvement in the 6MWT distance was missed. Recent evidence suggests that the minimum important clinical difference in the 6MWT distance in older people may be as low as 20 metres[378]. Even this more conservative value falls outside the 95% confidence intervals derived from the imputed data set. The lack of change in other measures of physical function including the TGUG, ISWT and FLP (a self-reported measure of physical function) with spironolactone reinforces our finding with the primary outcome.

4.1 Primary outcome

Several factors which may have contributed to the lack of effect of spironolactone on physical function:

Baseline six minute walk distance of study population

The mean baseline 6MWT distance was 340 metres, which was higher than previous studies involving frail older people[194;196]. This suggests that the study cohort was less impaired than those in previous studies. This may have been due to the fact that the majority of participants recruited were recruited from Primary Care, who were younger,

took less medication and required fewer walking aids than those who were recruited from Medicine for the Elderly services. A clinically significant improvement in exercise capacity may have been more difficult to detect in participants with a higher 6MWT distance. Pepin et al showed that COPD patients with a high mean baseline 6MWT distance were less responsive to change in exercise capacity following bronchodilator therapy compared to the endurance shuttle walk test[379]. It was suggested that the lack of responsiveness observed was related to a ceiling effect. However no ceiling effect was shown in this study.

Dosage of spironolactone

During the first 2 weeks, participants received 12.5 mg of spironolactone/ placebo and were then up-titrated to 25 mg for the remaining 18 weeks. The RALES study showed that 25 mg of spironolactone was pharmacologically effective at blocking mineralocorticoid receptors and 25 mg and was clinically effective at reducing morbidity and mortality in CHF patients[262]. Serious hyperkalaemia occurred most frequently at daily doses of 50 mg of spironolactone. However serious hyperkalaemia was only reported in 2% of patients in the RALES study in which the mean$\pm$SD age was 66$\pm$12 years compared to another study of spironolactone which showed that the hyperkaelamia and renal impairment occurred in up to 30% of patients aged over 75 years[380].

Spironolactone increases the risk of hyperkalaemia and renal impairment in older people[381]. The risk of having serious hyperkalaemia is much higher in older people receiving 50 mg of spironolactone or more[382 383]. To maintain participant safety in this study it was decided that a dose of 25 mg of spironolactone should not be exceeded.

Although it could be argued that the dose of spironolactone may have been insufficient to produce optimal pharmacological effects, this is probably not true for several reasons. Firstly, in contrast to previous studies, which reported higher rates of hyperkalaemia, participants in this study were excluded if they were already taking an ACE inhibitor and therefore their risks of hyperkalaemia were low to start with. Secondly, aldosterone levels were 60% higher with spironolactone indicating sufficient aldosterone blockade. Finally spironolactone increased both serum creatinine and potassium and reduced blood pressure at a similar rate to the RALES study where the dose of spironolactone was found to be pharmacologically effective[262]. This shows that the dose of spironolactone in this study was sufficient to provide pharmacological effects and that higher doses of spironolactone may have led to higher rates of hyperkalaemia without any further pharmacological benefit.

Adherence to study medication

Non-adherence to medication is often a major problem, with up to 59% of older people not taking their medication as prescribed[384]. Common reasons for non-adherence in older people include sensory deficits, cognitive problems, confusion over medication instructions or the belief that medication is not needed[385].

Adherence to medication was calculated by tablet-counting at each study visit. The median adherence in both the spironolactone and placebo group was 99%, higher than a randomised trial of multivitamins in community dwelling older people (91%)[386]. Many clinical trials classify good adherence between 80-90%, therefore this study achieved very good adherence in both groups[387]. However a higher proportion of participants in the spironolactone group (30%) took extra tablets during the study and one individual

had 120% adherence rate. Despite a higher proportion of participants in the spironolactone group taking extra tablets, this was not associated with an increase in adverse effects.

Measuring adherence through tablet-counting, makes the assumption that every tablet missing from the medication bottle is taken by the participant. Ideally adherence to medication should be measured by the 'gold standard' method of urine or serum analysis of the drug or a measurable metabolite related to the drug[388]. A significant rise in plasma aldosterone occurred with spironolactone, which supports a level of adherence to medication in the spironolactone group.

Power of the study

A final sample size of 120 participants provided a 80% power to detect a 30 metre change in distance walked in 6MWT, assuming a standard deviation of 50 metres at the 0.05 significance level, allowing for a 30% drop out rate. In reality 120 participants were recruited with only a 7% drop out rate, leaving 112 completers at 20 weeks. Despite spironolactone increasing the 6MWT distance by 31 metres (SD 79) the change in distance walked was 3 metres less than the placebo group at 20 weeks, which was not statistically significant. In this study the number of participants recruited was sufficient to detect a clinical effect in the primary outcome.

Other pharmacological effects of spironolactone

i) A rise in Angiotensin II (Ang II)

Spironolactone significantly increased aldosterone levels at 20 weeks ($p<0.01$). Although Ang II levels were not measured in this study, previous studies have shown that spironolactone is associated with a rise in both aldosterone and Ang II. The rise in aldosterone levels associated with spironolactone in our study was consistent with Rousseau *et al* who investigated neurohormonal levels in a subgroup of the RALES study[389]. Rousseau showed that spironolactone contributed to a significant increase in both aldosterone and Ang II levels after six months. It has been suggested that aldosterone blockade may cause a rise in aldosterone and Ang II through a positive feedback mechanism on the RAAS. Van de Wal *et al* showed that the use of spironolactone in CHF patients was an independent predictor of higher Ang II levels despite ACE inhibition[390]. In addition these patients also had higher plasma renin and Ang I levels with MR antagonism. It is possible that this rise in renin and Ang I associated with spironolactone may have induced a positive feedback on the RAAS thus forming more Ang II and aldosterone. Another suggestion is that the rise in Ang II with aldosterone blockade may also occur due to non-ACE conversion of Ang I to Ang II which has been shown in myocardial tissue, although the mechanism is unclear[391]. This could also explain why Ang II increases despite ACE inhibition in other studies.

High levels of Ang II are associated with negative cardiovascular effects and endothelial dysfunction[220]. Spironolactone has been shown to be associated with a rise in Ang II and poor endothelial function in type II diabetics without heart failure[250]. High levels of Ang II in animal models have been shown to induce muscle wasting and increase muscle protein degradation, which can ultimately lead to reduced muscle mass and

strength[229]. Therefore it could be speculated that a potential rise in Ang II with spironolactone, may have counteracted any potential benefit of aldosterone blockade on vascular endothelial function or skeletal muscle function.

ii) A reduction in androgen levels

Spironolactone has anti-androgen effects by binding to the androgen receptor and blocking the activation of dihydrotestosterone. The anti-androgenic effects of spironolactone are used as a treatment for hyperandrogenic disorders such as hirsuitism and acne vulgaris.

However these anti-androgenic effects may be associated with a detrimental effect on muscle function. Studies have shown that androgen replacement in hypogonadal men increases both muscle mass and strength[112]. One possible mechanism for sarcopenia in older people is low androgen levels which are commonly seen in older men. It could be speculated that the anti-androgen effects of spironolactone may have counteracted any potential benefits in terms of muscle mass and strength. As androgen levels were not measured in this study further research would be required to test this hypothesis.

iii) An increase in plasma cortisol

Spironolactone increases plasma cortisol levels[392]. Conditions of hypercortisolism, including Cushing's syndrome and medical treatments with glucocorticoid therapies are associated with muscle weakness and atrophy[393]. Cortisol causes a decline in muscle strength and causes muscle atrophy by inhibiting muscle protein synthesis[394]. The Longitudinal Ageing Study in Amsterdam showed an association between high salivary cortisol levels and a reduction in hand grip strength in older people[395]. More

importantly, high cortisol levels have been associated with poor physical function in old age.

Studies have shown that spironolactone increases plasma cortisol levels. When spironolactone binds to the mineralocorticoid receptor the clearance route for both mineralocorticoids and glucocorticoids is lost and hence the rise in available mineralocorticoid stimulates the glucocorticoid receptor. In addition, positive feedback may occur resulting in an increased production of ACTH and a rise in plasma cortisol secretion[396]. It could be suggested that the lack of effect of spironolactone on physical function may have been due to a rise in plasma cortisol, thus neutralising any beneficial effect of aldosterone blockade on skeletal muscle function.

In our study random plasma cortisol levels were measured at baseline and 20 weeks. A significant rise in plasma cortisol levels were seen in the spironolactone group in comparison to the placebo group in blood samples taken mid-afternoon. No significant correlation was found in our study between 6MWT distance and plasma cortisol levels at baseline. It could be speculated that the higher cortisol levels found within the spironolactone group may have contributed to deleterious effects on muscle function and offset any beneficial effect that may have occurred with aldosterone blockade. However the results from this study cannot confirm this hypothesis. As plasma cortisol levels fluctuate throughout the day with peak levels early morning (around 10am) and trough levels at night (around midnight), random cortisol results may not always be reliable. Our study was not designed to monitor changes in cortisol levels and further research would be required to assess this hypothesis.

Spironolactone is not effective at improving exercise capacity in the absence of heart failure

Spironolactone did not improve exercise capacity in our study. Whilst studies have shown an improvement in exercise capacity with spironolactone, the evidence exists in patients with heart failure[258]. As there was no improvement in exercise capacity in our study cohort of older people without heart failure, it could be assumed that the beneficial effects on exercise capacity with spironolactone are due to an improvement in cardiac function.

4.2 Secondary outcomes

There was no significant change between groups in the ISWT at 20 weeks. The mean ISWT distance at baseline was 228 metres which is in keeping with previous studies involving older patients[297;322].

In contrast to the 6MWT, not everyone was able to perform the ISWT at baseline. The ISWT was performed on the same day as the 6MWT therefore it is possible that participants were still recovering from the effects of the six-minute walk test. Dyer et al also showed in older (> 70 years) patients with COPD only 88% of participants were able to complete the ISWT at baseline mainly due to fatigue[325].

In our study, participants with walking aids found it particularly difficult to turn at either end of the course and subsequently led to them walking a shorter distance compared to those without walking aids. Previous studies have not highlighted this issue

as the participants selected to perform the ISWT were usually younger and fitter or had COPD who were limited by symptoms of dyspnoea and not immobility.

Singh et al showed the minimal clinically important difference in exercise capacity with the ISWT in COPD patients, was 47.5 metres[324]. In contrast, the change in ISWT distance between the spironolactone and the placebo group at 20 weeks was just 3.1 metres. The 95% confidence intervals do not include a change of 47.5 metres in the ISWT, therefore it is unlikely that a clinical effect was missed. However it must be remembered that this study was not powered to detect a change in ISWT distance. There was no significant change between groups in the time taken to perform TGUG. Unlike other field walking tests which measure endurance, the TGUG test is a useful measure of explosive muscle power required to get up out of the chair and walk at a quick pace. The mean TGUG of 13.3 seconds was similar to that reported in older people with established mobility problems, with 55% of participants taking >12 seconds to perform the test[315]. However, this result was lower than previous studies involving older patients at risk of falls (TGUG > 15 seconds)[315]. This shows that although participants had a moderate degree of physical impairment no significant change in muscle power was seen.

Spironolactone improved health related quality of life measured by EQ-5D. After adjusting for baseline EQ-5D score and age, spironolactone still improved EQ-5D by a clinically important difference[397]. Improvements in EQ-5D in older people have been associated with an improvement in 5-year survival[398].

The EQ-5D incorporates 5 domains in measuring quality of life including: mobility, usual activities, self-care, pain or discomfort and anxiety with depression. Subgroup analysis showed that of the 5 domains, there was a significant between group differences at 20 weeks within the pain domain, with 35% of participants in the spironolactone group reporting an improvement in pain score, with joint pain from osteoarthritis reported as the commonest cause of pain.

Spironolactone has been shown to have anti-inflammatory effects through suppression of pro-inflammatory cytokines. Previous animal studies have shown that aldosterone blockade reduces pro-inflammatory cytokine production and subsequent myocardial and vascular damage[220]. Spironolactone inhibits the production of key pro-inflammatory cytokines including TNF-α in studies of human blood leucocytes of patients with rheumatoid arthritis[254]. Aldosterone induces the activation of NADPH oxidase which is a major source of reactive oxygen species (ROS) and oxidative stress. This in turn also leads to an increase in pro-inflammatory cytokines such as TNF-α[254]. TNF-α also induces the production of other cytokines including IL-1α, IL-1β and IL-6 which are involved in numerous pathological inflammatory processes including arthritis. Therefore, it could be speculated that the improvement seen in quality of life with spironolactone may have been related to its potential anti-inflammatory effects. Further research is needed to test this hypothesis.

Unlike the EQ-5D there was no significant difference between treatment groups in change in the EQ-VAS score in this study at 20 weeks. There is conflicting evidence for the use of EQ-5D utility and EQ-VAS in measuring health state. EQ-5D is more useful at measuring change in health state in specific diseases such as Parkinson's disease and

rheumatoid arthritis which commonly lead to deteriorations in physical function[330;335]. The EQ-VAS has shown to be a better measure of overall health state and general well being. As the participants in this study were selected to have mobility problems this may explain the change in response in the EQ-5D utility and not EQ-VAS. This finding is in keeping with studies carried out involving a similar population with mobility problems[196].

The Functional limitation Profile (FLP) is a self-reported measure of the impact of illness on daily activity. There was no significant difference in FLP scores between the groups at 20 weeks. The lack of change in self-reported function supports the lack of change in physical function shown in our primary outcome.

Spironolactone was not associated with a significant change in psychological state as measured by the Hospital Anxiety and Depression Scale (HADS) questionnaire at either 10 or 20 weeks. In contrast, previous studies have suggested that high plasma aldosterone levels are associated with clinical depression[399] and spironolactone improved mood in women with premenstrual syndrome[400]. However, as the majority of participants in our study were euthymic any change in mood would be difficult to detect.

4.3 Changes in renal function and RAAS measurements

Participants had a mean baseline creatinine clearance of 88 ml/min which indicates mild renal impairment[401]. The baseline renal function in this study was better than the other longitudinal studies involving older people with multiple co-morbidities[402]. Spironolactone was associated with small but significant increases in serum potassium and creatinine with only a minor fall in sodium by 10 weeks. The changes in serum sodium, potassium and creatinine were similar to the changes found in the RALES study[262]. However, unlike the RALES study none of the participants had study medication discontinued due to serious hyperkalaemia (potassium >5.5 mmol/L). This was probably because participants in the RALES study were already taking an ACE inhibitor and had heart failure so therefore were at greater risk of serious hyperkalaemia. As expected, spironolactone was associated with a significant rise in plasma aldosterone, suggesting aldosterone blockade.

There was no significant change in serum magnesium between the two groups. This contradicts previous studies where spironolactone promoted magnesium retention by preventing magnesium excretion[230]. However this may be because the majority of studies involving spironolactone were conducted in patients with CHF, taking long-term magnesium losing diuretics and who had lower baseline magnesium levels.

BNP levels did not significantly change in either the spironolactone or placebo group at 20 weeks. There was also no correlation between change in 6MWT distance and change in BNP levels during the study in either group. Previous studies have suggested a

correlation between BNP levels and physical activities therefore the lack of change in BNP may reflect the lack of change in exercise capacity during the study[370].

4.4 Changes in Blood Pressure

Spironolactone was associated with a significant fall in both systolic and diastolic blood pressure at 10 weeks but did not cause postural hypotension and was well tolerated in a cohort of older people.

This was a smaller reduction in blood pressure compared to the Anglo-Scandinavian Cardiac Outcomes Trial Blood Pressure Lowering Arm (ASCOT-BPLA) study, where spironolactone was given to patients with resistant hypertension. The study showed a 21 mmHg reduction in systolic blood pressure and a 9 mmHg reduction in diastolic blood pressure with spironolactone. However it is difficult to extrapolate the results from the ASCOT-BPLA study, as these patients had resistant hypertension and without routine screening of secondary causes it is possible that some participants with hyperaldosteronism may have been recruited[403].

It has been suggested that the magnitude of blood pressure reduction is more important than the type of antihypertensive agent[404]. The reduction in diastolic blood pressure in this study was similar to results shown in the PROGRESS trial where a reduction in diastolic blood pressure of between 5 to 6 mmHg was associated with a 28% reduction in risk of stroke[405]. In a large meta-analysis involving 62,605 hypertensive patients a reduction in diastolic blood pressure of 5-6 mmHg was also associated with a 16% reduction in myocardial infarction[406].

Current British Hypertension Society guidelines recommend spironolactone as a fourth line therapy in patients with resistant hypertension[263]. This study indirectly boosts the case for using spironolactone as an antihypertensive in older people since it was well tolerated with associated reductions in blood pressure.

4.5 Recruitment

The recruitment of frailer older people to a clinical trial is difficult. Our study population represented only 3.5% of the total population screened; however this was in keeping with previous randomised controlled trials in frailer older people[407]. A total of 87% of potential participants contacted by letter failed to reply, with a greater proportion of non-responders coming from those attending the Medicine for the Elderly services. This may be because patients were attending the Day Hospital or clinics in Medicine for the Elderly department for an ongoing medical problem and therefore may be unwell or reluctant to take additional medication. Also the letters sent to those in Primary Care were signed by their own GP's which may have encouraged more people to reply, if their GP was happy for them to participate.

Twenty three percent of potential participants did not meet inclusion criteria. The main reason for individuals from the community not meeting inclusion criteria was if they had no problems with activities of daily living. Individuals who volunteer for research may be healthier which may only represent one end of the spectrum of the general older population.

Through review of participant demographics we believe our study cohort is representative of a frail older population. Comorbid disease is prevalent in older people.

Participants in the study had a range of comorbid diseases including hypertension (29%), COPD (19%) and Parkinson's disease (5%). Given that participant selection focused on participants with mobility impairment it was not surprising that 45% of participants had osteoarthritis. Rates of ischaemic heart disease and diabetes mellitus were lower than community dwelling older people recruited to the Cardiovascular Health Study[282]. This is probably because ACE inhibitors and angiotensin receptor blockers are commonly prescribed in these patients. However in this study participants were excluded if they were taking these medications.

The majority of patients within our study cohort, were recruited from a Primary Care setting. Although mean 6MWT distance in our study cohort was similar to other studies involving community dwelling older people[300], and older people with mobility limitations[408] it was higher than previous studies assessing exercise capacity from our department[194;196]. The mean TGUG was 13.3 seconds which is in keeping with results from other studies which include older people with mobility problems but it is was lower than frail older residential people with a high falls risk[316]. Participants in this study walked 70 metres further at baseline in the ISWT compared to previous studies involving older patients[325]. However the majority of studies using the ISWT involve patients with COPD, who are mainly limited by breathlessness when performing the test rather than mobility problems.

4.6 Strengths and limitations

As a parallel group randomised controlled trial, participants and investigator were blind to treatment. All data were entered and the analysis of the primary outcome performed prior to breaking the treatment code. All outcomes were measured by a single investigator. Outcomes were measured using Standard Operating Procedures without reference to results from previous visits, thus eliminating both investigator and participant bias.

Data entry and analysis was performed independently by the Robertson Centre for Biostatistics, Glasgow. Data were entered by the data manager and the database was audited on several occasions in order to prevent data entry errors. Data were analysed using intention-to-treat analysis in order to minimise any deviation from randomised allocation or missing responses, which may have occurred due to participants not completing the study.

This study had several limitations. Firstly, the use of self-reported problems in activities of daily living, by using the Barthel Index, as a means of assessing functional status. Traditionally self-reports or proxy-reports have proved useful in assessing functional ability of older people as they are easy to administer and inexpensive, however self-reports measure perceived function and may have introduced subject bias. The Barthel Index is also limited by ceiling and floor effects which may limit its responsiveness to change[409]. Sinoff et al showed that the self-reported Barthel ADL Index was found to have limitations in the very old (>85 years old) and in patients with cognitive impairment[410]. Measuring physical performance by direct observation may have provided a more objective way to measure the participants functional state[411]. Physical

performance tests such as the 'sit-to-stand tests' have been shown to be useful predictors of disability[412]. Giampolini et al showed that hand grip strength is a good predictor of disablity; with a reduction in grip strength associated with increased disability at 4 years after adjusting for cofounders including osteoarthritis[413].

Secondly, although the 6MWT has been widely used to measure of submaximal exercise capacity there is a lack of consensus regarding the definite reference ranges of the test. Although in general a distance of less than 300 m is considered the threshold for functional impairment, previous studies show discrepancies in the 'normal ranges' for the 6MWT varying from 383-820 m[414]. The variation of threshold distances between studies may be due to differences in the proctocols used for the 6MWT and whether or not encouragement was given during the test. Participants may also stop and rest during the test which may have influenced the distance walked during the study as well as the tests responsiveness to change with intervention.

Thirdly an apparent 'learning effect' was seen in the 6MWT in both groups at 20 weeks. No practice tests were performed prior to measuring the 6MWT distance at each visit. Studies have shown that the 6MWT distance tends to increase after repeated test administration, therefore producing a learning effect because of test familiarisation[414]. The learning effect seen in previous studies varies in magnitude from 4.5-33% of the initial distance, however the distance plateaus after the third repeat[299]. The study showed a learning effect in both groups at 20 weeks, with each group showing a 9% increase in distance walked from baseline. Although studies in COPD and CHF patients have suggested good reproducibility of the 6MWT after only one test, relatively few

studies have performed reliability studies of the test in older people. Kervio examined the reliability of the 6MWT in healthy older people and found that similarly at least two familiarisation tests were required to eliminate a learning effect[415]. Therefore, it is possible that the learning effect may have been mitigated if participants had performed at least 2 practice walks. However as this study included a frail older population with established mobility problems practice tests in the 6MWT may have been difficult to accomplish.

Although results of the RAAS measures were only available at the end of the study, the investigator monitored blood results for participants' renal function and magnesium levels at each study visit. Knowledge of the participants' potassium or creatinine levels may have led to unblinding of the investigator resulting in investigator bias. However it is important to note that meticulous monitoring of blood results was essential to ensure patient safety. This ensured that in the event of a participant developing serious hyperkalaemia immediate action would be taken by the investigator. In an ideal situation this would have been avoided by employing an additional staff member to monitor participants' renal function independently from the research team.

Randomisation

Randomisation was carried out by Tayside Pharmaceuticals, using a computer generated random numbers table. As randomisation was performed independently from the study team, treatment allocation was concealed. Treatment codes were placed in a sealed envelope and were held by Tayside Pharmaceuticals. After the study was completed and all data were entered, allocation to treatment group 'A' or 'B' was performed by a

person independent of the research team. Treatment codes were only broken after analysis of the primary outcome was performed.

Blinding

The following strategies were implemented in order to preserve blinding within the study: Participants were randomised and the treatment allocation was concealed from both the participant and investigator throughout the study which prevented the introduction of bias to the study. Results of BNP and aldosterone were only available at the end of the study. Analyses of these measures were performed at the end of the study to prevent investigator unblinding.Data were entered onto the computer database by an independent organisation and the database was 'locked' prior to the treatment codes being broken. Data analysis of the primary outcome was performed blind.

4.7 Adverse events

There were no serious adverse events in our study associated with spironolactone. Two participants were hospitalised prior to commencing their study medication due to unrelated medical problems. Only 7% of participants withdrew from the study. This was much lower compared to the RALES study where 25% of participants were withdrawn and Witham *et al* showed that up to 42% of older patients with heart failure were withdrawn from taking spironolactone due to adverse effects[416]. There were no episodes of serious hyperkalaemia in our study cohort.

The majority of participants tolerated the up-titration of spironolactone to 25 mgs after 2 weeks. Only 3% of individuals in the spironolactone group were down-titrated to 12.5 mg spironolactone due to a rise in potassium, which was much lower than participants

in Cicoira's study, where 8% of participants in older people with heart failure were down titrated to 12.5 mg of spironolactone due to adverse effects[258].

4.8 Conclusion

This study showed that spironolactone did not improve exercise capacity in older people without heart failure. Previous studies suggested that spironolactone may improve physical function by preventing skeletal myocyte loss[225], improve vascular endothelial function[248] by improving skeletal muscle blood flow[249] and improve muscle contractility by increasing muscle magnesium and potassium[231]. However the majority of these studies were performed in animals or in patients with CHF, where improvements in physical function were more likely due to improvements in cardiac function. The fact that ACE inhibitors did improve exercise capacity in functionally impaired older people without heart failure supports the role of other inhibitors of the RAAS in preventing sarcopenia. However the lack of effect with spironolactone shows that blocking aldosterone alone does not prevent decline in physical function with age.

Spironolactone was associated with improvement in health related quality of life, despite having no effect on exercise capacity. Previous studies with spironolactone have shown improvements in quality of life in association with improvements in exercise capacity or cardiac symptoms in patients with CHF[258;262]. To the best of our knowledge this was the first study to show an improvement in quality of life in older people without heart failure in the absence of any improvement in physical function. The most significant improvement in quality of life was in the pain domain of the EuroQol EQ-5D, with joint pain from osteoarthritis as the commonest cause of pain. It is possible that improvement in quality of life may have occurred due to other pharmacological effects of spironolactone including an anti-inflammatory effect. Previous studies have

shown spironolactone to have anti-inflammatory potential as it can reduce pro-inflammatory cytokines, which are stimulated in a range of inflammatory disorders including arthritis. This observation suggests possible wider pharmacological effects of spironolactone in older people.

Current British Hypertension Society guidelines recommend spironolactone as a fourth line therapy for resistant hypertension[263]. Our findings support findings by Wei *et al*[266] that spironolactone is a safe drug to use if closely monitored[266]. It also provides reassurance that spironolactone was generally well tolerated with relatively few adverse effects and no postural hypotension in a frail older population.

5. FUTURE RESEARCH

ACE inhibitors and spironolactone on exercise capacity

The ACE inhibitor perindopril improved exercise capacity in functionally impaired older people without heart failure at 20 weeks[196], however there is a lack of evidence to suggest whether this improvement in physical function can be sustained with long term ACE inhibition. High levels of angiotensin II and aldosterone have a negative impact on muscle function. Levels of angiotensin II and aldosterone fall within the first 10 months of taking an ACE inhibitor. However observational studies have shown that aldosterone levels eventually return to baseline with the long term use of ACE inhibitors due to 'aldosterone escape'[257]. The subsequent rise in aldosterone may counteract the initial benefits of ACE inhibitors on exercise capacity. The initial improvement in exercise capacity may be sustained long term with the addition of aldosterone receptor blocker, spironolactone.

Spironolactone and health related quality of life

Spironolactone improved quality of life in functionally impaired older people without heart failure. Further randomised placebo controlled trials involving spironolactone would be useful to investigate whether an improvement in quality of life could be generalised in a wider older population.

In this study spironolactone was associated with improved pain score in the EuroQol EQ-5D, especially in participants with osteoarthritis. Osteoarthritis is one of the

commonest causes of disability in older people, mainly affecting the weight-bearing joints. Older people with symptomatic osteoarthritis are more likely to have problems with activities of daily living, poorer quality of life and increased risk of falls.

Although the EuroQol EQ-5D questionnaire is a valid measure of change in general health state in chronic conditions including arthritis it is less responsive to moderate changes in pain score, which makes it more difficult to relate these findings to the general population. Randomised placebo-controlled trials are now required to investigate whether spironolactone improves pain as measured by validated measurement tools which have been used to assess pain in older osteoarthritis patients i.e. the pain Visual Analogue Scale (VAS)[417].

Spironolactone and inflammation

One theory for the improvement in quality of life, in particular the improvements seen in the pain domain of quality of life, could be due to the potential anti-inflammatory properties of spironolactone. Studies have shown that spironolactone inhibits inflammatory cytokine production in patients with arthritis and reduces vascular inflammation[254]. In older people chronic inflammation is a strong predictor of physical disability and mortality[16]. Further studies are needed to investigate the anti-inflammatory potential of spironolactone and the possible long-term benefits on morbidity and mortality in older people.

6. PUBLICATIONS AND PRESENTATIONS

Publications

Burton LA, Sumukadas D. Optimal management of sarcopenia. *Clinical Interventions in Aging. 2010 Sep 7;5:217-28*

Burton LA, McMurdo MET, Struthers AD. Mineralocorticoid antagonism: a novel way to treat sarcopenia and physical impairment in older people. *Clinical Endocrinology 2011* Jun 23. doi: 10.1111/j.1365-2265.2011.04148.x. [Epub ahead of print].

Oral Presentation

Burton LA. Effect of spironolactone on exercise capacity in functionally impaired older people without heart failure: a double blind placebo controlled trial. *University of Dundee, Postgraduate Research Symposium. June 2010*.

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8. APPENDICES

Appendix A - Participant screening proforma

Patient no……

Exclusion Criteria

On spironolactone ☐ Age <65 years ☐ Uncontrolled hypertension ☐

Heart Failure ☐ Creatinine >200mmol/L/ eGFR <30ml ☐ Addisons disease ☐

Serum Na <130mmol/L ☐ Potassium >5.0mmol ☐ MMSE <15/30 ☐ wheelchair

bound ☐ systolic blood pressure <100mmHg ☐ On ACE inhibitor ☐

On Angiotensin Receptor Blocker ☐ In another trial / within 3 months of completing

another trial ☐ symptomatic postural hypotension ☐ living in nursing home ☐

Inclusion Criteria

Age > 65 years ☐

Functional disability: Walking aids ☐

Help with ADL ☐

Functional State Self reported / discharge from Medicine for the Elderly Services

a) Mobility: no aids ☐ one aid ☐ two aids ☐ frame ☐ rollator ☐

b) Manages stairs: yes ☐ with help ☐ no ☐

c) Toileting : independent ☐ needs help ☐

d) Dressing: independent ☐ minimal help ☐ needs help ☐

e) Grooming (hair/teeth/shave): independent ☐ needs help ☐

f) Bathing: independent ☐ needs help ☐

g) Kitchen: capable ☐ snack only ☐ incapable ☐ not applicable ☐

h) Feeding: independent ☐ needs help ☐

i) Bowels: continent ☐ occasional accident ☐ incontinent ☐

j) Bladder: continent ☐ catheter ☐ urodomes ☐ occ.accident ☐

incontinent ☐

Eligible to take part in study: YES ☐ NO ☐

Study Information given: YES ☐ NO ☐

Date…… **Signature……..**

Appendix B – Letter of invitation

Ageing and Health
Division of Medical Sciences
University of Dundee
DD1 9SY
Tel: 01382 632436
Date: / /

Dear Mr/Mrs,

Research Project: Effect of Spironolactone On Exercise Capacity In Older People Without Heart Failure:
A Double Blind Placebo Controlled Trial

We are delighted to invite you to take part in our research project. We are interested in seeing whether a medicine called spironolactone, commonly used in patients with heart failure could help to improve your muscles. Improving muscles can help to reduce the risk of disability.

We are approaching you about this research because you have recently attended the Medicine for the Elderly services at Perth Royal Infirmary and do not have heart failure.

Full details of what the study is about and what would be involved are enclosed in the Patient Information Leaflet. If you are interested, we would be delighted to hear from you and can arrange to meet you to answer any questions you may have. Please kindly fill in the attached reply slip if you are interested in hearing more about the research and send it back to us in the enclosed stamped addressed envelope.

In the meantime, please feel free to contact us by telephone on Dundee 632436 during office hours if you have any queries.

Yours sincerely

Dr Louise Burton
Clinical Research Fellow
University of Dundee

Professor Marion E T McMurdo
Head of Section of Ageing and Health
University of Dundee

Appendix B – letter of invitation

Reply slip: Effect of spironolactone on exercise capacity in older people

Only complete if you wish to learn more about the study

Name……..…….

Address……....……...……...

…….

…….……...……..… Postcode …….....

Telephone number……....……

Best time to contact……..……

Appendix C – Participant Information Leaflet

PATIENT INFORMATION SHEET

Effect Of Spironolactone On Exercise Capacity In Older People Without Heart Failure: A Double Blind Placebo Controlled Trial

We invite you to participate in a research project. We believe it to be of potential importance. However, before you decide whether or not to participate, we need to be sure that you understand firstly why we are doing the study, and secondly what it would involve if you agreed to take part. We are therefore providing you with the following information. Please take time to read it carefully and be sure to ask any questions you have, and, if you want, discuss it with others. We will do our best to explain and provide any further information you may ask for now or later. You do not have to make an immediate decision.

Why are we doing this study?
Recent evidence suggests that a medicine called spironolactone that is commonly used in patients with heart failure could have a beneficial effect on muscle function. This improvement in muscle function can lead to decreased effort in performing day to day activities and increase exercise capacity even in people without heart failure.

We are doing this study to find out if spironolactone will improve improve muscle function in older people without heart failure.

Why have I been chosen?
You have been chosen for this research because you are over 65 years old.

Do I have to take part?
Participation in this study is entirely voluntary and you are free to refuse to take part or to withdraw from the study at any time without having to give a reason and without this affecting your future medical care or your relationship with medical or nursing staff looking after you.

What will happen to me if I take part?
The study will last for 5 months and you will be given either the spironolactone or placebo (dummy) capsules. You have a 50/50 chance of receiving either treatment.

You will be requested to come to the Royal Victoria Hospital in Dundee or Perth Royal Infirmary, three times over these 5 months. We will arrange a free taxi to bring you to the Hospital and take you back home each time. Each visit will last between one and two hours.

You will be asked to perform some walking tests and asked to answer a few questionnaires.

We will take down details of what medicines you are taking.

We will measure your blood pressure and take some blood samples (no more than a few teaspoonfuls).

We will do an echocardiography scan of your heart. This is a scan similar to that done on pregnant women to look at their babies.

What do I have to do?
You are required to take the medication (1 capsule per day) every day for 5 months.

We will ask you to do the following at each visit:

- Walk up and down a corridor for six minutes. You may rest on the way as many times as you need to.
- Walk around a short course in time to a beeping sound for as long as you can.
- Get out of a chair and walk a few paces. We will time you.
- Answer three questionnaires about how much you are able to do and how you feel.

What is being tested?
We want to test whether those who receive the spironolactone during the study improve in their walking and other tests compared to those who received the placebo. This would give us an idea whether the medicine has effects on physical function in older people.

Will taking part in the study affect your usual care?
We will not alter any of your medication or interfere with your treatment in any way.

What are the possible discomforts, risks and side effects?
Spironolactone is a diuretic therefore you may experience an increase in urine output, an upset stomach, dizziness or kidney problems with increases in blood levels of potassium. Therefore your potassium levels will be monitored throughout the study. Men may experience possible breast enlargement.The blood test may cause some minor discomfort.

Taking part in this study could lead to mild tiredness.

What are the possible benefits of taking part?
Those of you who receive the spironolactone may improve your muscle function and exercise capacity. This may potentially reduce disability and dependence.

What happens if something goes wrong?
This is very unlikely, but if something does go wrong then the University of Dundee has a scheme which could provide compensation, if appropriate.

Will my GP know about this research project?
If you agree to take part, we will inform your GP.

Will my taking part in this study be kept confidential?
All the information collected will be kept strictly confidential. All information will be kept in a locked room and held on a secure computer. During the study all identifying information will be kept separately, and at the end of the study your name and address will be deleted. Only the researchers involved in the study will have access to the information. We will keep the information for 15 years.

What will happen to the results?
The results will be examined by the researchers and a report produced. You will not be identified in this report. The results will be shared with the funder for the study (Scottish Chief Scientist Office), and be added to the growing evidence on the effects of these medicines. We will write to let you know the results at the end of the study.

Will I continue to receive the medicine used in this study after it finishes?
You will not continue to receive the medicine after the study finishes.

Who is organizing and funding this research?
The study has been organized by Prof Marion McMurdo, Dr Deepa Sumukadas, Dr Miles Witham and Prof Allan Struthers. The study is funded by the Scottish Chief Scientist Office

Who has reviewed the study?
The Tayside Committee on Medical Research Ethics, which has responsibility for scrutinising all proposals for medical research on humans in Tayside, has examined the proposal and has raised no objections from the point of view of medical ethics. It is a requirement that your records in this research, together with any relevant medical records, be made available for scrutiny by monitors from NHS Tayside and the Regulatory Authorities.

Thank you for reading this Information Sheet and considering taking part in this study.

For further information contact:

Dr Louise Burton, Research fellow, on telephone Dundee 632436.

Dr D Sumukadas on telephone Dundee 632436

Professor M E T McMurdo on telephone Dundee 632436

Appendix D – Consent form

Study Number: 08/S1402/34
Patient Identification Number for this trial:

CONSENT FORM v1.3 Dated 16/11/09

Title of Project: **The effect of spironolactone on exercise capacity in functionally impaired older people without heart failure: a double blind placebo controlled trial**

Name of Researcher: Professor Marion E T McMurdo

Please initial or tick box

1 I confirm that I have read and understood the information sheet dated 21/10/2009 (version 1.3) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2 I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without any medical care or legal rights being affected ☐

3 I understand that relevant sections of my medical notes and data collected during the study may be looked at by regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4 I agree to my GP being informed of my participation in the study. ☐

5 I agree to the storage of my leftover blood samples for use in future research. ☐

6 I agree to take part in the above study. ☐

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Name of person taking consent	Date	Signature

When complete, 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes

Appendix E- Letter to GP

Ageing and Health
Division of Medical Sciences
University of Dundee
DD1 9SY
Ph: 01382 632436
Fax: 01382 660675
Date: --/--/--

Dr XYZ
GP Surgery

Dear Dr,

Re: Mr/Mrs ABC, address

Effect Of Spironolactone On Exercise Capacity In Older People Without Heart Failure: A Double Blind Placebo Controlled Trial

Mr/Mrs ABC has very kindly agreed to take part in the above trial. Recent research suggests that modifying the renin-angiotensin system may have a role in improving exercise capacity in older people.

We are running this trial to assess whether spironolactone can improve exercise capacity in older people without heart failure. Patients will be randomised to spironolactone or placebo for a 20 week period. We will provide all of the trial medication. Both the patients and the assessors will be blinded to the nature of the therapy. All other medications can continue as usual. We will assess walking distance, ability to get out of a chair, quality of life and exercise capacity at the beginning, halfway through, and at the end of the study. We will also check blood pressure, serum sodium, potassium, urea, creatinine and magnesium at 0, 2, 5, 10 and 20 weeks. Portable echocardiography will be done at the beginning and the end of the study.

The project is funded by the Scottish Chief Scientists Office. The study has been approved by the Tayside Committee on Medical Ethics. If you have any questions about the study, or if there is any aspect of your patient's healthcare that we can assist you with, please do not hesitate to call us on the numbers below.

Yours sincerely,

Dr Louise Burton
Clinical Research Fellow
Ageing and Health
University of Dundee

Professor Marion ET McMurdo
Head of Ageing and Health
University of Dundee

Appendix F – Mini mental state questionnaire

Mini Mental State Exam (MMSE)

Date: …… Study Number: ……

ORIENTATION

Year	Month	Day	Date	Time	____ / 5
Country	Town	District	Road	Number	____ / 5

REGISTRATION

Examiner names 3 objects (e.g. apple, table, penny)
Patient asked to repeat (1 point for each correct)
Patient to learn the 3 names repeating until correct ____ / 3

ATTENTION AND CALCULATION

Subtract 7 from 100, then repeat from result
Continue 5 times: 100 93 86 79 65
Or
Spell W O R L D backwards – dlrow ____ / 5

RECALL

Ask for names of 3 objects learned earlier ____ / 3

LANGUAGE

Name a pencil and a watch ____ / 2

Repeat “No ifs, ands or buts” ____ / 1

Give a 3 stage command. Score 1 point for each stage
Take this piece of paper, fold it in half, drop it on the floor ____ / 3

Ask patient to read and obey a written command on a piece of paper stating “Close your eyes” ____ / 1

Ask patient to write a sentence. Score if it is sensible and has a subject and a verb ____ / 1

COPYING

Ask patient to copy a pair of intersecting pentagons ____ / 1

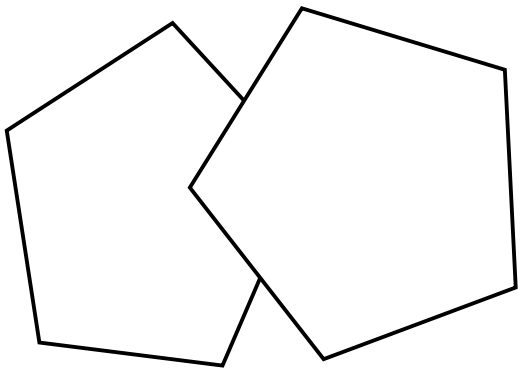
TOTAL ____ / 30

Close your eyes

……..

Write a Sentence: _____

……



Appendix G – EuroQol (EQ-5D Utility section) questionnaire

Date: …… Study Number: …… Visit Number:…….
By Placing a tick in one box in each group, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about ☐

I have some problems in walking about ☐

I am confined to bed ☐

Self-care

I have no problems with self-care ☐

I have some problems washing or dressing myself ☐

I am unable to wash or dress myself ☐

Usual activities (e.g. work, study, housework, family or leisure activities)

I have no problems performing my usual activities ☐

I have some problems with performing my usual activities ☐

I am unable to perform my usual activities ☐

Pain / Discomfort

I have no pain or discomfort ☐

I have moderate pain or discomfort ☐

I have extreme pain or discomfort ☐

Anxiety / Depression

I am not anxious or depressed ☐

I am moderately anxious or depressed ☐

I am extremely anxious or depressed ☐

Appendix H– EuroQol (EQ-VAS section)

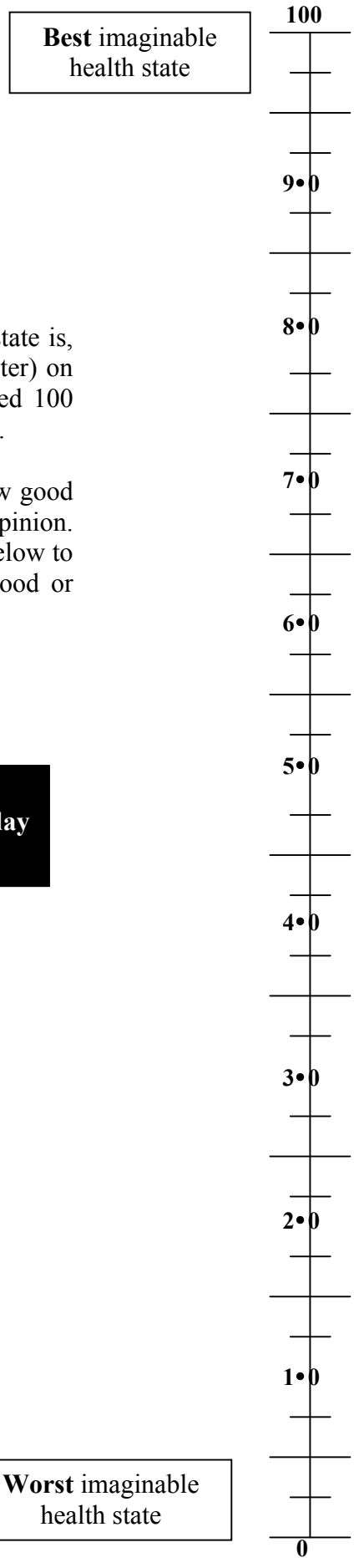
Date: ……

Study Number: ……

Visit Number: ……..

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.



Appendix I – HADS questionnaire

Patient study number:	

Effect Of Spironolactone On Exercise Capacity In Older People Without Heart Failure: A Double Blind Placebo Controlled Trial

Hospital Anxiety and Depression Score

Read each question and tick the box that is closest to how you have felt **over the last month:**

		Score	
Do you feel tense or 'wound up':	☐ Most of the time	3	**A**
	☐ A lot of the time	2	
	☐ From time to time, occasionally	1	
	☐ Not at all	0	
Do you still enjoy the things you used to enjoy:	☐ Definitely as much	0	**D**
	☐ Not quite so much	1	
	☐ Only a little	2	
	☐ Hardly at all	3	
Do you get a sort of frightened feeling as if something awful is about to happen:	☐ Very definitely and quite badly	3	**A**
	☐ Yes, but not too badly	2	
	☐ A little, but it doesn't worry me	1	
	☐ Not at all	0	
Can you laugh and see the funny side of things:	☐ As much as I always could	0	**D**
	☐ Not quite so much now	1	
	☐ Definitely not so much now	2	
	☐ Not at all	3	
Worrying thoughts go through your mind:	☐ A great deal of the time	3	**A**
	☐ A lot of the time	2	
	☐ From time to time, but not too often	1	
	☐ Only occasionally	0	
Do you feel cheerful:	☐ Not at all	3	**D**
	☐ Not often	2	
	☐ Sometimes	1	
	☐ Most of the time	0	
Can you sit at ease and feel relaxed:	☐ Definitely	0	**A**
	☐ Usually	1	
	☐ Not often	2	
	☐ Not at all	3	

Patient study number:	

Do you feel as if you are slowed down:

- ☐ Nearly all the time 3 **D**
- ☐ Very often 2
- ☐ Sometimes 1
- ☐ Not at all 0

Do you get a sort of frightened feeling, like 'butterflies' in the stomach:

- ☐ Not at all 0 **A**
- ☐ Occasionally 1
- ☐ Quite often 2
- ☐ Very often 3

Have you lost interest in your appearance:

- ☐ Definitely 3 **D**
- ☐ I don't take as much care as I should 2
- ☐ I may not take quite as much care 1
- ☐ I take just as much care as ever 0

Do you feel restless as if you have to be on the move:

- ☐ Very much indeed 3 **A**
- ☐ Quite a lot 2
- ☐ Not very much 1
- ☐ Not at all 0

Do you look forward with enjoyment to things:

- ☐ As much as I ever did 0 **D**
- ☐ Rather less than I used to 1
- ☐ Definitely less than I used to 2
- ☐ Hardly at all 3

Do you get sudden feelings of panic:

- ☐ Very often indeed 3 **A**
- ☐ Quite often 2
- ☐ Not very often 1
- ☐ Not at all 0

Can you enjoy a good book or radio or TV program:

- ☐ Often 0 **D**
- ☐ Sometimes 1
- ☐ Not often 2
- ☐ Very seldom 3

Score: **D ……. / 21**

A ……. / 21

Signature:……

Date……

Appendix J – Functional Limitation Profile

FUNCTIONAL LIMITATIONS PROFILE

Ambulation items

The following statements describe walking and use of stairs. Remember, think of yourself today.

1	I do not walk at all		126
2	I get about in a wheelchair		121
3	I do not use stairs at all		106
4	I only walk with help from someone else		98
5	I get about only by using a walking frame, crutches, stick, walls or hold onto furniture		96
6	I only go up and down stairs with assistance from someone else		87
7	I only use stairs with a physical aid; for example a handrail, stick or crutches		82
8	I walk by myself but with some difficulty; for example, I limp, wobble or stumble, or I have a stiff leg		71
9	I do not walk up or down hills		64
10	I go up and down stairs more slowly; for example one step at a time, or I often have to stop		62
11	I walk shorter distances or I often stop for a rest		54
12	I walk more slowly		39
	SCORE	**/126**	

Body care and movement items

The following statements describe how you move about, bath, go to the toilet, dress yourself today.

13	I am in a restricted position all the time		124
14	I do not have control of my bowels		124
15	I do not have control of my bladder		122
16	I stay lying down most of the time		120
17	I use a bedpan with help		107
18	I do not bathe myself at all; but am bathed by someone else		100
19	I do not get in and out of bed or chairs without the help of a person or mechanical aid		100
20	I do not keep my balance		93
21	I only stand up with someone's help		93
22	I do not bathe myself completely; for example, I need help with bathing		85
23	I make difficult movements with help; for example getting in or out of the bath or car		82
24	I hold onto something to move myself around in bed		82
25	I only get dressed with someone's help		82
26	I get in and out of bed or chairs by grasping something for support or by using a stick or walking frame		79
27	I spend most of the time partly dressed or in pyjamas		75
28	I do not fasten my clothing; for example I require assistance with buttons, zips or shoelaces		68
29	I only stand for short periods of time		67
30	I move my hands or fingers with some difficulty or limitation		66
31	I kneel, stoop or bend down only by holding onto something		61
32	I have trouble putting on my shoes, socks or stockings		54
33	I change position frequently		53
34	I am very clumsy		47
35	I dress myself, but do so very slowly		43
	SCORE	**/124**	

Mobility Items

These next statements describe how you get about the house and outside

36	I stay in bed most of the time		114
37	I stay in one room		101
38	I stay in bed more		91
39	I stay at home most of the time		79
40	I only get about in one building		76
41	I only go out if there is a lavatory nearby		64
42	I do not get about in the dark or in places that are not lit unless I have someone to help		57
43	I do not use public transport now		52
44	I do not go into town		47
45	I only stay away from home for short periods		46
	SCORE	**/ 114**	

Household management items

The following statements describe your daily work around the home. When you answer, think of yourself today.

46	I do not do any of the daily household chores that I would usually do		90
47	I do not do any of the shopping that I would usually do		84
48	I do not do any of the cleaning that I would usually do		78
49	I have difficulty using my hands; for example turning taps, using kitchen gadgets, sewing or doing repairs		78
50	I do not do any of the maintenance or repair work that I would usually do in my home or garden		75
51	I do not do any of the clothes washing that I would usually do		75
52	I have given up taking care of personal or household business affairs; for example paying bills, banking or doing household accounts		69
53	I do not do heavy work around the house		59
54	I only do housework or work around the house for short periods of time and I rest often		50
55	I do less of the daily household chores than I would usually do		37
	SCORE	**/ 90**	

Recreation and Pastime items

The following statements describe the activities you usually do in your spare time, for relaxation, entertainment, or just to pass the time. Again, think of yourself today.

56	I am not doing any of my usual inactive pastimes; for example. I do not watch TV, play cards, or read		91
57	I am not doing any of my usual physical recreation or more active pastimes		81
58	I am cutting down on some of my usual inactive pastimes; for example I watch TV less, play cards less or read less		50
59	I am doing more inactive pastimes instead of my other usual activities		43
60	I am cutting down on some of my usual physical recreation or more active pastimes		34
61	I spend shorter periods of time on my hobbies and recreation		32
62	I go out less often to enjoy myself		27
63	I take part in fewer community activities		25
	SCORE	**/ 91**	

Social Interaction items

These statements describe your contact with family and friends today.

64	I refuse contact with my family; for example I turn away from them		109
65	I frequently get angry with my family; for example I hit them, scream or throw things at them		103
66	I isolate myself as much as I can from the rest of my family		100
67	I do not go out at all to visit people		91
68	I stay alone much of the time		91
69	I am disagreeable with my family; for example I act spitefully or stubbornly		86
70	I make many demands on other people; for example I insist that they do things for me or tell them how to do things		76
71	I avoid having visitors		73
72	I do not look after my children or family as well as I usually do		66
73	I am often irritable with those around me; for example I snap at people or criticise easily		64
74	My sexual activity is decreased		64
75	I pay less attention to the children		59
76	I show less interest in other people's problems; for example, I don't listen when they tell me about their problems; I don't offer to help		50
77	I show less affection		44
78	I often express concern over what might be happening to my health		44
79	I talk less with other people		44
80	I do not joke with members of my family as much as I usually do		38
81	I go out less often to visit people		31
82	I am cutting down the length of visits with friends		31
83	I take part in fewer social activities than I used to; for example I go to fewer parties or social events		25
	SCORE	**/ 109**	

Emotion Items

The next statements describe your feelings and behaviour. Again, think of yourself today

84	I have attempted suicide		141
85	I talk hopelessly about the future		96
86	I say how bad or useless I am; for example that I am a burden on others		89
87	I am irritable and impatient with myself; for example, I run myself down, I swear at myself, I blame myself for things that happen		79
88	I often moan and groan because of pain or discomfort		67
89	I keep rubbing or holding areas of my body that hurt or are uncomfortable		59
90	I laugh or cry suddenly		58
91	I get sudden frights		56
92	I behave nervously or restlessly		48
	SCORE	**/ 141**	

Alertness items

93	I sometimes get confused; for example I do not know where I am, who is around, or what day it is		115
94	I have more minor accidents; for example I drop things, I trip and fall, or I bump into things		90
95	I forget a lot; for example things that have happened recently, where I put things, or to keep appointments		85
96	I have difficulty reasoning and solving problems; for example making plans, making decisions, or learning new things		78
97	I am confused and start to do more than one thing at a time		74
98	I have difficulty doing things which involve thought and concentration		71
99	I do not keep my attention on any activity for long		52
100	I react slowly to things that are said or done		52
101	I make more mistakes than usual		49
102	I do not finish things I start		45
	SCORE	**/ 115**	

Sleep and Rest items

These statements describe your sleep and rest activities today

103	I sleep or doze most of the time, day and night		111
104	I spend much of the day lying down to rest		96
105	I sleep less at night; for example I wake up easily, I don't fall asleep for a long time, or I keep waking up		86
106	I sit around half asleep		84
107	I sleep or doze more during the day		80
108	I lie down to rest more often during the day		72
109	I sit for much of the day		62
	SCORE	**/ 111**	

Eating items

The following statements describe your eating and drinking habits

110	I eat no food at all except by tubes or intravenous infusion		143
111	I do not feed myself at all but have to be fed		121
112	I eat no food at all, but I take liquids		113
113	I feed myself with help from someone else		95
114	I feed myself but only with specially prepared food or special utensils		76
115	I eat special or different food; for example I follow a soft food, bland, low fat, low salt or low sugar diet		52
116	I just pick or nibble at my food		39
117	I eat much less than usual		34
118	I drink less fluids		33
	SCORE	**/ 143**	

Communication items

I am going to read out some statements about how much you talk to other people and write. Please think about yourself today

119	I communicate mostly by nodding my head, pointing or using sign language or other gestures		127
120	My speech is understood only by a few people who know me well		94
121	I am understood with difficulty		89
122	I don't write except to sign my name		84
123	I speak with difficulty; for example, I get stuck for words, I stutter, I stammer, I slur my words		76
124	I often lost control of my voice when I talk; for example my voice gets louder or softer or changes unexpectedly		59
125	I carry on a conversation only when very close to other people or looking directly at them		59
126	I have trouble writing or typing		50
127	I do not speak clearly when I am under stress		47
	SCORE	**/ 127**	

Work items

I am going to read out some statements about work. As I read them out, think of yourself today. If today is not a working day for you, think about your last working day

128	I do not work at all		361
129	I only work for short periods of time or often stop to rest		65
130	I only do light work		56
131	I work shorter hours		52
132	I do not do my job as carefully and accurately as usual		50
133	I often get irritable with my workmates; for example I snap at them or criticise them easily		42
134	I am not getting as much work done as usual		41
135	I do part of my job at home		40
136	I work at my usual job but with some changes; for example I use different tools or special aids or I swap jobs with someone else		36
	SCORE	**/361**	

TOTALS:

Ambulation	/126	Recreation	/ 91
Body care	/124	Social	/ 109
Mobility	/114	Emotion	/ 141
Household	/ 90	Alertness	/ 115
		Sleep / rest	/ 111
Physical domain total:	**/ 454**	**Psychosocial domain total:**	**/ 567**
Eating	/ 143		
Communication	/ 127		
Work	/ 361	**GRAND TOTAL:**	**/ 1652**